Metal removal processes in wetlands receiving acid mine drainage.

Lesley Claire Batty
Dept. of Animal and Plant Sciences, University of Sheffield.

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Eriophorum angustifolium in a wetland receiving AMD.

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This study provides information on the processes active within wetlands that remove potentially phytotoxic metals from drainage waters, including rhizospheric processes with direct reference to plaque formation on the roots of wetland plants.

In natural wetlands receiving AMD, processes of metal sulphide and oxide formation together with adsorption onto organic matter were found to be more significant than metal uptake into plant tissues. However, plants influenced the sediment environment through the addition of organic matter, oxidation of the rhizosphere and increasing the retention time of contaminated waters thereby affecting the active chemical processes. This was shown to be important both spatially and temporally within wetlands.

Root plaques were successfully formed both under laboratory and field conditions at low (3.5) and high (6.0) pH. Plaque deposits were composed of iron, manganese or aluminium oxides depending upon the chemical environment. Iron and aluminium plaques were found to reduce but not prevent the uptake of Cu, Mn, Al and Zn. Previous reports of metal adsorption onto the plaque surface were shown to be an artefact of the extraction technique and did not cause the inhibition of metal uptake. An alternative theory was proposed, suggesting that the production of H^+ ions during plaque formation competed with metals for binding sites and thus reduced their movement into plant tissues. This effect was amplified at low pH when high concentrations of H^+ ions were present in the growth medium.

Seasonal variations in plaque formation were found, with dissolution of iron plaques on roots of *T. latifolia* during the winter months. Equivalent release of Fe did not occur in *P. australis* due to venturi-flow through dead culms.

The implications of these results are discussed in relation to the use of constructed wetlands within the water treatment industry.

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1. General Introduction

Both active and abandoned mine sites are a major source of toxic metals and acidity which may affect wildlife, water and land resources. Wastewaters originating from these sites are difficult to treat as they often fluctuate in volume, have high metal loads, are poorly buffered, have extreme pH and low levels of organic compounds (Dunbabin & Bowmer 1992). Wetlands (both natural and constructed) have been used since the 1970's to remove potentially toxic metals from contaminated drainage (Stillings *et al.* 1988; Eger & Lapakko 1989; Wildeman & Laudon 1989; Lan *et al.* 1992; Dodds-Smith *et al.* 1995; Beining & Otte 1996; Scholes *et al.* 1998). The majority of research into the role of wetlands has concentrated on their ability to remove metals rather than on the active processes that occur within the wetland system (e.g. Tarleton *et al.* 1984; Stillings *et al.* 1988; Wieder 1993). It has become evident, however, that an understanding of the chemical, physical and biological processes is essential if wetlands are to be used to their maximum potential. Recent investigations have therefore concentrated on the individual sinks for metals including the sediments, vegetation and surface waters. The extent to which each sink removes metals and the importance of interactions between them remain unclear.

The work reported in this thesis seeks to assess the relative importance of rhizospheric processes in the removal of heavy metals from acid mine drainage in the context of the whole ecosystem using a combination of field and laboratory techniques. This introductory chapter outlines the chemical, physical and biological environments that occur within wetland systems. Emphasis will be placed on the root-sediment interface where a number of processes are active. The chapter will conclude with an evaluation of current knowledge and the specific aims and objectives of this thesis.

1.1 The Wetland Environment

1.1.1 Physical and chemical characteristics

A key definition of wetlands has remained elusive, however for most scientific purposes that defined by the U.S. Fish and Wildlife Service 1979 (cited in Hammer & Bastian 1989) is sufficient. Wetlands are required to meet one or more of three conditions:-

a) Areas supporting predominantly hydrophytes (at least periodically)

b) Areas with predominantly undrained hydric soil (wet enough for long enough to produce anaerobic conditions that limit the types of plants that can grow there)

c) Areas with non-soil substrate (such as rock or gravel) that are saturated or covered by shallow water at some time during the growing season.

Wetland soils are characterised by anaerobic conditions induced by flooding. Oxygen becomes deficient within a few centimetres of the soil surface as oxygen diffusion in flooded soils is nearly 10,000 times slower than in aerobic soils (Armstrong 1978). Once flooded, any oxygen present is consumed rapidly by the metabolism of microbes and chemical oxidation (Howeler & Bouldin 1971). Once this oxygen is consumed, anaerobic micro-organisms utilise a series of alternative electron acceptors during respiration. These include such oxidised components as nitrate, manganese dioxide, hydrated oxides of iron (III), sulphate and their own metabolites (Ponnamperuma *et al.* 1967). This process produces an overall reduction in the redox potential of the soil through the conversion of some chemical species into a state of reduction. Nitrate is the first soil component to be reduced after oxygen at a redox potential, assuming neutral pH, of 220 mV. This is followed by manganic manganese (Mn^{4+}) at 200 mV, ferric iron at 120 mV, sulphate from -75 to -150 mV and carbon dioxide between -250 and -300 mV. The overall reactions are shown in Table 1.1.

Oxidised State	Reduced State
$O_2 + 4H^+ + 4e^-$	$2H_2O$
$NO_3^- + 2H^+ + 2e^-$	$NO_2^- + H_2O$
$Mn^{4+} + 4H^+ + 2e^-$	$Mn^{2+} + 2H_2O$
$Fe^{3+} + 2H^+ + 2e^-$	$Fe^{2+} + 3H_2O$
$SO_4^{2-} + 10H^+ + 8e^-$	$H_2S + 4H_2O$
$CO_2 + 8H^+ + 8e^-$	$CH_4 + 2H_2O$

Table 1.1. Reaction sequence of chemical reduction

Although the reduction reactions of nitrate and manganic manganese may occur concurrently, the subsequent reactions will not unless the preceding component has been completely reduced. Flooding of soils also affects the pH, with a general trend towards neutrality. This is probably due to the build-up of carbon dioxide in alkaline soils, and due to the reduction of ferric oxide in acid soils as follows

$$3Fe(OH)_3 + H^+ + e^- \Leftrightarrow Fe_3(OH)_8 + H_2O$$

As a result of the reduction of a variety of compounds including iron (III) and manganese (IV) as well as copper and nickel, the reduced forms can be released into soil solution and become increasingly bioavailable. In addition, iron reduction may release phosphate from the dissolution of reductant-soluble P, a poorly crystalline iron compound which is stable under oxidised conditions (Faulkner & Richardson 1990). Reduction of sulphate to sulphide (H_2S) however, may reduce the solubility of elements such as iron, zinc and cadmium by the formation of sparingly soluble sulphides (Marschner 1995).

1.1.2 Wetland Vegetation

Plants growing in flooded soils are faced with an environment deprived of oxygen and rich in soil nutrients which may reach toxic levels. Wetland plants have developed a variety of physiological adaptations to enable growth in this stressed environment. Due to the anoxic nature of the soil, oxygen requirements of the roots must be met by movement of oxygen from the aerial part of the plant to subaerial parts. The development of lacunae and/or aerenchyma is a characteristic of non-woody species which increases the porosity of the plant by as much as 60% (Armstrong 1976). This allows movement of oxygen to the root zone which has been measured at a rate between 2.08 g O_2 m^2 d^{-1} (Brix & Schierup 1990) and 5 to 12 g O_2 m^2 d^{-1} (Armstrong *et al.* 1990) in *Phragmites australis*. Initially it was thought that this process was achieved through gas-phase convection and/or diffusion. However, aeration of the rhizome system is enhanced through convective flow driven by humidity and temperature gradients (Armstrong & Armstrong 1988, 1990 a & b, 1991; Armstrong *et al.* 1992). Although loss of oxygen from the root is reduced by the formation of exodermis, oxygen still diffuses out from the roots particularly from young adventitious, secondary roots and basal regions of laterals (Armstrong & Armstrong 1988). This diffusion of oxygen has been termed radial oxygen loss (R.O.L). The resulting oxidation of the rhizosphere has been implicated in the removal of phytotoxic concentrations of organic solutes and iron and manganese from the surrounding soil solution (Marschner 1995; Peverly *et al.* 1995).

In addition to R.O.L., wetland plants may also develop shallow root systems in the aerated upper part of the soil (Black 1968) in order to obtain oxygen.

1.2 Iron Plaque Formation

Coatings of iron (oxyhydr-)oxides on the roots of plants have been termed 'iron plaques'. These plaques are present as an orange-brown deposit on the root surface. They have been recorded on the roots and rhizomes of monocotyledons, dicotyledons and non-flowering plants subjected to flooding including *Oryza sativa, Typha latifolia, Spartina* spp., and *Phragmites australis*. (e.g. Greipsson 1994; Snowden &

Wheeler 1995; Ye *et al.* 1998a; Sundby *et al.* 1998; Wang & Peverly 1999). In order for iron plaques to form, two conditions are necessary: a soluble form of iron in the wetland substrate and locally oxidising conditions. The exact cause of iron precipitation around roots however remains unknown but three main hypotheses have been proposed.

1.2.1 Radial Oxygen Loss

As a result of the diffusion of oxygen from the roots of wetland plants, oxidising conditions occur in a confined area around the root. Due to the saturated status of wetland soils the chemical components including iron become chemically reduced (Figure 1.1). At the root-sediment interface the oxygen reacts with the reduced iron (and manganese) which is oxidised and precipitates out as a deposit on the root surface. However this explanation is not entirely satisfactory. Research has shown that there is no correlation between R.O.L. and plaque formation (Armstrong 1967). In addition R.O.L. was found to account for only 1/9th of the oxidation in the rhizosphere of *Molinia caerulaea* and *Menyanthes trifoliata* (Armstrong 1967). It is therefore evident that direct oxidation by diffusing molecular oxygen may account only in part for root oxidising activity.

1.2.2 Root exudates

The release of oxidants other than molecular oxygen from the root may be important in root oxidising activity. Extracts from the roots of *Oryza sativa* have been found to be capable of oxidising Fe^{2+} to Fe^{3+} (Yamada & Ota 1958). The active enzyme involved has been suggested to be glycolic acid oxidase (Mitsui *et al.* 1962) but this has yet to be proven. It has been suggested that such enzyme activity may account for up to 90% of the total oxidation (Armstrong 1976).

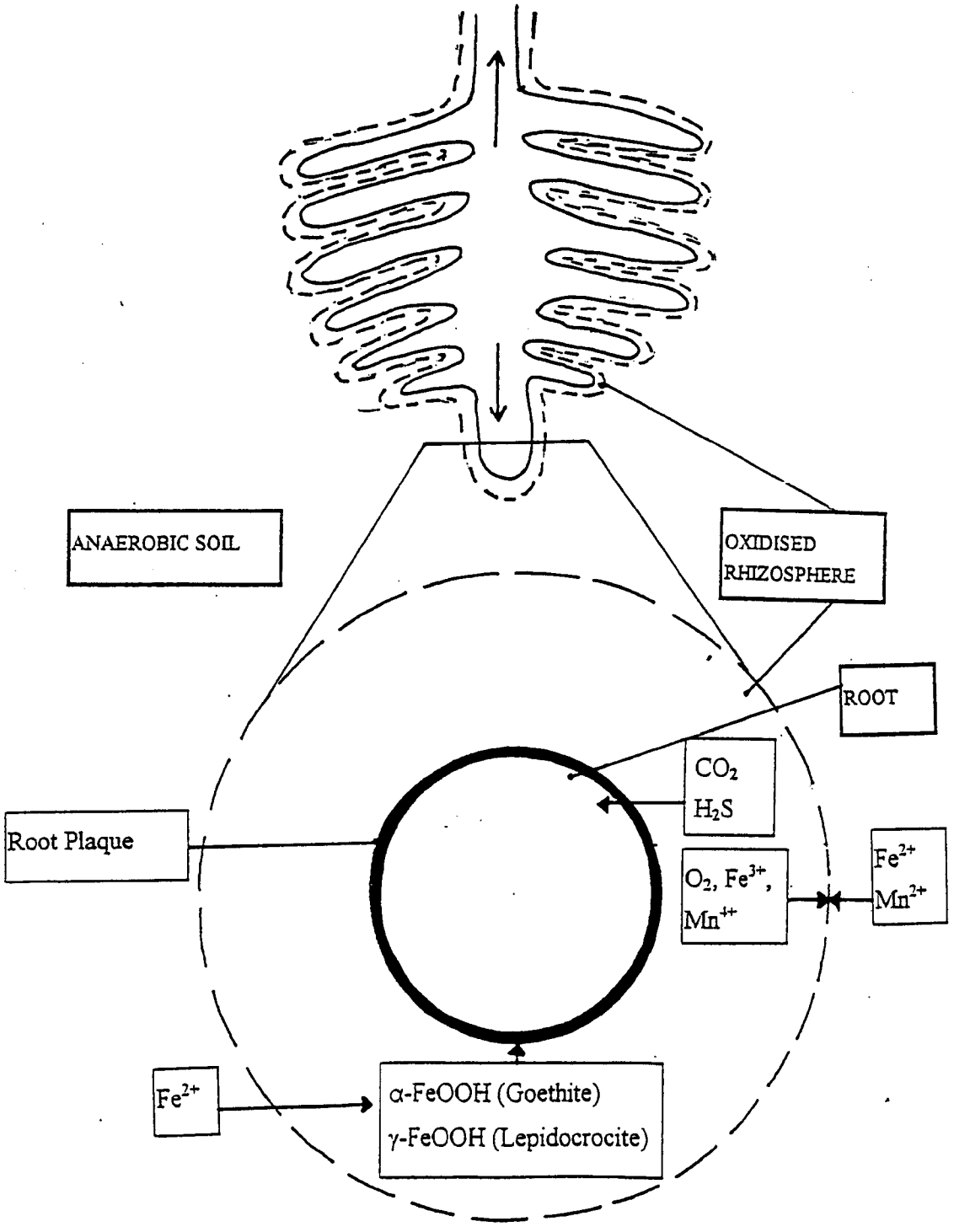


Figure 1.1 Formation of iron oxyhydroxide plaques as mediated by R.O.L.

1.2.3. Rhizospheric Bacteria

Iron oxidation may be mediated by the presence of micro-organisms including bacteria and fungi. Microbially mediated iron oxidation followed by precipitation of iron oxyhydroxides as the orange sludge, 'yellow boy' within sediments has been well documented. The micro-organism *Thiobacillus ferrooxidans* is the most active former of bog iron ore and is known to catalyse the oxidation of Fe^{2+} at pH's less than 3.5 (Batal *et al.* 1989).

Spherical structures resembling bacterial colonies have been observed in the oxidised rhizosphere of *Oryza sativa* (Trolldenier 1988). Reddish brown colonies developed when suspensions of these spherical structures were transferred to iron rich agar. It is unknown however, whether these colonies are of iron oxidising bacteria. Gram negative and gram positive bacilli have also been found in plaque (Crowder *et al.* 1987), and micro-organisms have been noted in the plaques of *Vallisneria americana* Michx. (St Cyr *et al.* 1993). This research also suggested that filamentous bacteria may act as a focus for iron precipitation by increasing surface area and thereby accelerating the depositional rate of iron (St Cyr *et al.* 1993). In contrast to these findings however, Johnson-Green & Crowder (1991) demonstrated that iron-oxidising bacteria and/or other micro-organisms were not required for iron plaque formation. The presence of bacteria was thought to reduce the formation of plaques by the consumption of oxygen, altering the permeability of the root surface and by the complexation of Fe^{2+} by bacterial exopolysaccharides. The precise role of micro-organisms in rhizospheric oxidation therefore, remains unclear.

1.3 Characteristics of plaques

1.3.1 Occurrence

The distribution of plaque on root surfaces is highly variable. In general, the plaque is patchy in its coverage of the root surface and varies in thickness (St Cyr *et al.* 1993). However, in some cases distinct patterns of zonation are reported but these differ with plant species and stage of root development (Chen *et al.* 1980a; Taylor *et al.* 1984; Otte *et al.* 1989; Cook 1990; Snowden & Wheeler 1995). At a cellular level, the

penetration of iron precipitates into root tissues has been shown to vary. Mendelssohn & Postek (1982) reported that deposits did not penetrate the outer cell wall of the root surface of *Spartina alterniflora* Loisel. In contradiction to this, Levan & Riha (1986) found iron and manganese deposits present within the cell walls of the root epidermis and cortex.

Plaque has also been found as fillings of exposed cavities or as complete hollow polyhedra (Chen *et al.* 1980b, Taylor *et al.* 1984). Two modes of development have been proposed for these forms of plaque. Firstly, the deposition of iron within exposed cavities following the decomposition and collapse of the tangential cell wall and secondly, precipitation of iron inside epidermal cell walls prior to the decomposition of the plant tissues. Both of these processes are described in detail in Chen *et al.* 1980b.

1.3.2 Mineralogy and chemical composition

Iron oxide deposits are generally seen as an irregular porous coating on the root surface (Bacha & Hossner 1977, St Cyr *et al.* 1993). On a microscopic scale the plaque may be either amorphous or crystalline. Amorphous plaque has been identified on plaques formed under both field conditions and in solution culture (Taylor & Crowder 1983; McCarthy 1985 cited in Crowder & St Cyr 1991). Crystalline deposits have been reported to form in the field on the roots of *Vallisneria americana* (St Cyr *et al.* 1993) as a mixture of dispersed amorphous material, nodules, needles and filaments. Lath-shaped crystals have also been observed in an electron dense matrix on the roots of *Oryza sativa* (Chen *et al.* 1980a). Plaque deposits have been analysed using a variety of techniques including x-ray diffraction and energy dispersive x-ray microanalysis. The coatings have been reported to consist of lepidocrocite (γ-FeOOH) (Bacha & Hossner 1997), a mixture of lepidocrocite and goethite (α-FeOOH) (Chen *et al.* 1980a; St Cyr *et al.* 1993) and ferric phosphate (Snowden & Wheeler 1995).

Although iron plaques are predominantly composed of iron, manganese may also form plaque deposits. Manganese oxide is usually found in conjunction with iron oxide but often at a higher concentration than iron (Bacha & Hossner 1977; Mendelssohn *et al.*

plants experience a shortage in iron supply from the rooting medium (Bienfait *et al.* 1984, 1985). In locations where water is stagnant, conditions during the summer can lead to the unavailability of iron due to lowering of the water table and subsequent aeration of the soil.

1.4.2. Reservoir of nutrients

In addition to iron, plaques may also contain a number of accessory elements including nutrients and may therefore act as a reservoir for these essential elements (Trolldenier 1988; Conlin & Crowder 1989). It has been demonstrated that concentrations of phosphorus, sulphur and magnesium are higher in plants of *Typha latifolia* and *Carex rostrata* with an iron plaque than those without (Crowder *et al.* 1987) which indicates that plaque increases nutrient uptake. In the same report however, it was noted that phosphorus, sulphur and potassium concentrations were lower in field-formed plaques and therefore nutrient deficiency may result from adsorption in a nutrient poor environment. This was also suggested for *Oryza sativa* (Howeler 1973).

Phosphorus is generally considered to be the most important nutrient in this hypothesis. It has been identified within root plaques (St Cyr *et al.* 1993) and is known to form iron-phosphate complexes (Snowden & Wheeler 1995). The relationship between iron and phosphate is also well documented in the chemical field (e.g. Kuo 1986). The oxidation of iron results in the production of acidity as hydrogen ions are released. This acidification may help to solubilise nutrients such as phosphorus in times of deficiency (Begg *et al.* 1994). This has also been proven for zinc (Kirk & Bajita 1995) which is often deficient in alkaline, organic or poorly-drained soils.

1.4.3 Barrier to phytotoxic elements

In flooded environments the reduction of iron and manganese compounds results in the accumulation of high levels of these bioavailable elements. In addition, particularly in acid soils, there may be potentially phytotoxic levels of metals or metalloids. Wetland species have been observed to grow and flourish in highly

contaminated environments and these plants frequently possess iron plaques. Iron oxides present in soils and sediments have high specific surface areas and possess -OH functional groups which are capable of reacting with metals and other cations and anions (Kuo 1986). It is possible that iron hydroxides forming on the roots of wetland plants have similar properties and may therefore immobilise and prevent the uptake of phytotoxic metals.

This 'exclusion' hypothesis is supported by reports of the amelioration of the toxic effects of phytotoxic metals in a variety of species. Greipsson & Crowder (1992) and Greipsson (1994) reported that the presence of iron plaque on *Oryza sativa* seedlings improved growth under mildly toxic conditions of copper and (or) nickel exposure. However, iron plaque also appeared to enhance the uptake of iron and it was proposed that the high concentration of iron in the leaves enabled competition by iron with copper for sensitive metabolic sites (Greipsson 1994). Crowder *et al.* (1987) also found that the growth of *Oryza sativa* exposed to 0.5 ppm copper was also greater in the presence of plaque. In contrast, investigations into the growth of *Typha latifolia* demonstrated that the presence of plaque on the roots of seedlings did not enhance metal tolerance or growth when subjected to copper and (or) nickel, zinc and (or) lead and cadmium (Ye 1995; Ye *et al.* 1997a, b, 1998a). The presence of plaque however did reduce root length. Extensive research has also been undertaken to determine whether the presence of plaque reduces the uptake of phytotoxic metals.

Iron plaques have been shown to act as a filter for metal movement into rhizomes and shoots for iron, copper, zinc, nickel and cadmium (Greipsson & Crowder 1992; Greipsson 1994; Wang & Peverly 1995). Otte *et al.* (1987, 1989) also found that iron plaque may act as a barrier to zinc for the species *Aster tripolium* L., but only at low external zinc concentrations. At higher external levels, the plaque appeared to enhance the uptake of zinc. In contrast, the majority of research has shown that the formation of plaque does not impede the uptake of toxic metals. This has been demonstrated for the uptake of iron in *Oryza sativa* (Benckiser *et al.* 1984), manganese in *Picea mariana* Mill. and *Pinus resinosa* Ait. (Levan & Riha 1986), manganese in *Oryza sativa* (Crowder & Coltman 1993), copper and zinc in *Phragmites australis* (St Cyr & Crowder 1987), copper and nickel in *Typha latifolia* (Ye *et al.* 1997a) and zinc and lead in *Typha latifolia* (Ye *et al.* 1998a).

It is evident from this that, to date, we have conflicting information and the precise role of plaque remains unresolved. The research that has been undertaken has included investigation of seedlings from a wide variety of species including freshwater, saltwater and non-wetland plants, together with the use of material from both the field and the laboratory. In the controlled conditions of the laboratory a number of techniques have been used with differing growth media including nutrient solutions, sand and agar, a variety of pH conditions, differing metal sources which has already been proved to be an important factor (Taylor *et al.* 1984) together with a number of different analytical techniques. It is therefore difficult to compare different investigations and to extract any definitive conclusions from the literature.

1.5 Acid Mine Drainage

Acid mine drainage (AMD) results from the exposure of sulphide minerals to water and air which causes the oxidation of sulphide to sulphate and in turn produces sulphuric acid. The increased acidity lowers the pH of the environment and this decreases adsorption and hence increases mobility of metals in soils, sediments and water (Alloway & Ayres 1993). The majority of research has focused on the generation of AMD from pyrite (FeS_2) but may also result from other sulphide minerals. The pathways and reactions of pyrite oxidation are as follows

$$FeS_2 + {}^7/_2\, O_2 + H_2O \Rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$$

$$Fe^{2+} + {}^1/_4\, O_2 + H^+ \Rightarrow Fe^{3+} + {}^1/_2\, H_2O$$

$$Fe^{3+} + 3H_2O \Rightarrow Fe(OH)_{3\,(s)} + 3H^+$$

$$FeS_{2\,(s)} + 14Fe^{3+} + 8H_2O \Rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

The oxidation of pyrite is often catalysed by microbes which can increase the rate of reaction by five to six orders of magnitude. These obligately acidophilic bacteria include *Thiobacillus thiooxidans* which oxidises reduced sulphur and ferrous iron, and *Leptospirillum ferrooxidans* which oxidises ferrous iron (Walton & Johnson 1992).

The pH and metal content of AMD is dependent upon the amount of sulphide and acid-consuming minerals present, and the balance between the processes of sulphide oxidation and acid consumption. AMD is a major cause of contamination problems in the UK and throughout the world (Plates 1.1 & 1.2). The metal mining industry has exposed extensive areas of sulphide minerals resulting in the production of large quantities of AMD. Due to the location of many mines close to water courses which were used as an energy source, the contamination of these waters has been extensive and continues due to the lack of reclamation.

Many techniques have been developed to treat mine spoil and AMD which either restrict the supply of oxygen and/or water to pyritic ores or inhibit the bacteria that catalyse the oxidation of sulphides. In the UK reclamation methods include

- covering of surface waste with uncontaminated capping material
- concentration of waste in one place prior to capping
- revegetation with metal tolerant plants
- excavation, removal and disposal off site
- on site reprocessing
- reducing discrete water inputs to mine works

(NRA 1992)

In addition to these, the discharge has also been treated using a variety of chemicals including sodium hydroxide, calcium hydroxide, calcium oxide, sodium carbonate and ammonia. Discharge is also frequently directed through limestone drains, all of which act to neutralise the pH of the drainage waters and to encourage the precipitation of metals. The main problem with these techniques is that they tend to be very expensive due to the cost of the chemicals, operation and maintenance costs and additional expense associated with the disposal of metal laden sludges. This is combined with

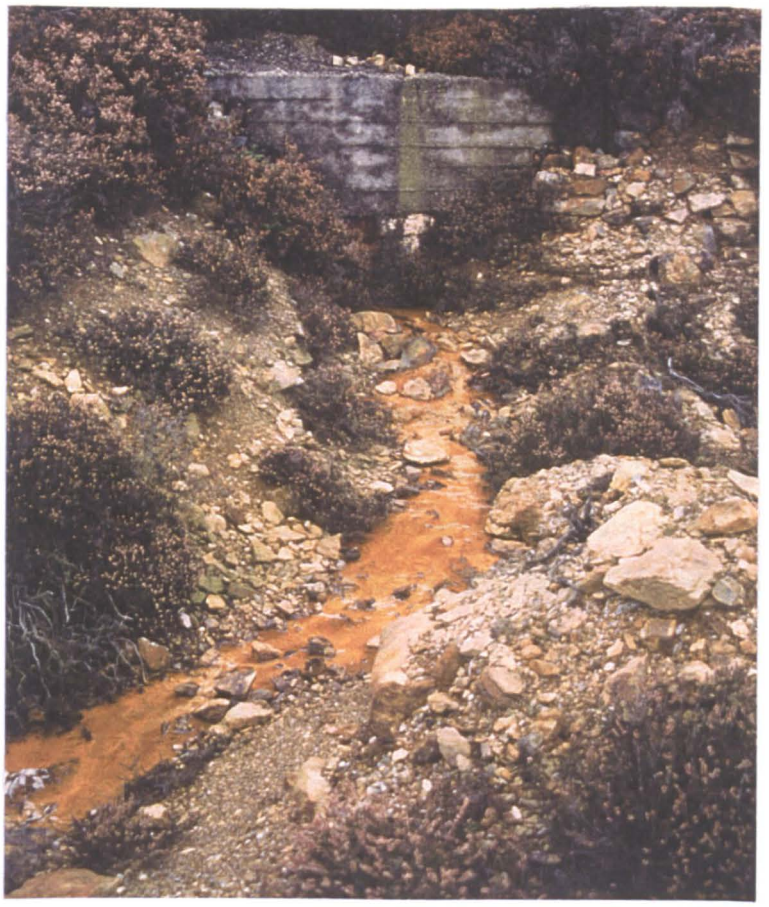


Plate 1.1. Acid mine drainage at Parys Mountain

Plate 1.2. Acid mine drainage 3 km downstream from Parys Mountain.

problems with the techniques themselves, for example limestone drains frequently suffer from armouring of surfaces with iron precipitates which reduces their effectiveness. As an alternative to these labour intensive methods, passive technologies including the use of wetlands have been developed and refined in recent years. Initially the research concentrated on the possible uses of *Sphagnum* spp. (e.g. Tarleton *et al.* 1984), but this was found to be unsuccessful due to its intolerance of iron and fluctuating water depth. Alternative designs were investigated and this has led to the use of emergent species such as *Typha latifolia* which have been found to be more successful. Research has since concentrated on the capacity of wetlands to remove metals and the relative importance of the various immobilisation processes that occur.

It has generally been found that experimental and field systems have been successful in removing metals from both acid and neutral drainage (Tarleton *et al.* 1984; Griffin *et al.* 1989; Bolis *et al.* 1991; Puckett *et al.* 1993; Eger 1994; Peverly *et al.* 1995; Stark *et al.* 1995 a, b; Tam & Wong 1996; Mungur *et al.* 1997; Scholes *et al.* 1998). In addition, wetlands have been shown to be effective in removing high levels of nutrients such as phosphorus and nitrogen from wastewater (Wolverton *et al.* 1983; Reddy & De Busk 1985; Gersberg *et al.* 1986; Peverly *et al.* 1995; Mandi *et al.* 1996). Wetlands can also increase pH to a more neutral condition (Bolis *et al.* 1991; Eger 1994). The processes of metal removal in wetlands tend to be biogeochemical processes, the most significant of which are:

- sorption and/or exchange onto organic matter
- formation of carbonates
- association with iron and manganese oxides
- metal hydrolysis
- reduction to non-mobile forms
- formation of insoluble metal sulphides

The relative importance of these processes varies with environmental conditions. The formation of organically-bound iron was found to be the most important in *Sphagnum* dominated microcosms (Tarleton *et al.* 1984). However, in many cases the formation

of iron oxides has been identified as the primary process of iron removal in wetland systems (Griffin *et al.* 1989; Henrot & Wieder 1990; Wieder *et al.* 1990; Stark *et al.* 1995a; Tarutis & Unz 1995). The formation of sulphides, oxides and carbonates constitute the most stable form of trace element precipitates. Although these processes are predominantly geochemical, the presence of plants in wetland systems is extremely important in the removal of metals from contaminated discharge. The presence of vegetation has been shown to improve the removal of contaminants in experimental systems (Wolverton *et al.* 1983). Macrophytes are significant in the stabilisation of the sediment surface, provide conditions suitable for physical filtration of suspended and colloidal material and provide a large surface area for microbial growth. Microbes are important in both the reduction of metals and in metal hydrolysis. Although macrophytes such as *Phragmites australis* and *Typha latifolia* have been shown to take up contaminants including metals (Lan *et al.* 1992; Scholes *et al.* 1998; Zhu *et al.* 1999), the removal of metals via this pathway is unlikely to be important on the larger scale. Sencindiver & Bhumbla (1988) found that *Typha latifolia* removed less than 1% of the total iron added to a wetland system.

1.6 Conclusions

This chapter has highlighted the significance of wetland systems in the removal of metals and other substances from water. This has important implications in the reclamation of contaminated areas and the prevention of large scale pollution incidents resulting from the release of AMD. The relative importance of the presence of wetland plants however remains unclear. The growth of macrophytes in these highly contaminated areas has led to extensive research into the adaptations of such species to extremely high levels of metals. The presence of iron oxide plaques has been invoked as a preventative mechanism to limit the uptake of these potentially phytotoxic metals. The precise role of root plaques is not fully understood and detailed knowledge of the rhizospheric processes involved has not been attained. In order to utilise constructed wetlands to their maximum efficiency it is essential that our understanding of wetland biogeochemical processes is improved and this needs to

include an investigation into the interactions that occur between the vegetation, porewaters, surface waters and soils/sediments.

1.7 Aims & Objectives

The main aim of this study therefore is to assess the role of plant species in the removal of metals within wetland systems. Within this there are a number of objectives that need to be achieved:-

- identify the major sinks of metals within contaminated wetlands.
- identify the processes involved in iron plaque formation on roots of wetland plants.
- characterise the plaques formed in both the laboratory and the field.
- determine the role of plaques in the removal of potentially phytotoxic metals from contaminated systems.

2. General methods and materials.

2.1 General methodologies for plant materials

2.1.1 Collection and storage of seeds

It was necessary to have a consistent source of *Phragmites australis* seed for all experiments and therefore seeds were collected from an uncontaminated (non-wetland) field site, located in Felixstowe, U.K The seeds were collected in 1997 and kept in the dark at room temperature in sealed polythene bags until required.

2.1.2 Seed Germination

The germination of *Phragmites australis* seeds for experiments reported in Chapters 6 & 7 required specific conditions. Seeds were first removed from inflorescences by vigorous rubbing between the hands. They were then placed in a shallow plastic tray, fully imbibed with UHP water and kept in the dark, in a refrigerator at 4°C for a period of 14 days. At the end of this time the seeds were transferred to a plastic sandwich box (28 x 16 x 9 cm) containing a layer of alkathene® beads together with 1L of 10% Rorison's nutrient solution (appendix A). The seeds were sprinkled on top of the beads, the box covered with a clear lid and then placed in a controlled environment room (16 hr 20°C day, 80 μmol m^{-2} s^{-1} photon flux density, 8 hr, 14°C night) for approximately 7 days or until germination was evident. The lid was then removed and the seedlings allowed to grow for a further 2 weeks until reaching a size suitable for transplantation. During this time the nutrient solution was replaced every 3 days.

2.1.3 Preparation of plant samples for analysis

All plant samples were rinsed thoroughly in UHP water to remove dust and sediments/soils contaminating their surfaces. Each plant was then divided into roots and shoots. The shoots were carefully blotted dry and sealed in envelopes for drying at 40°C until constant weight was reached, usually after 3d, and finally cooled to room temperature in a desiccator and stored there until required. At this time, shoots were

ground to a homogeneous sample in a knife mill and stored in clean polythene bags while grinding was completed for up to 1 hr before digestion.

Roots were subjected to two different extraction techniques in sequence.

DCB extraction

Metals adsorbed or loosely bound to the root surface were extracted using the DCB (dithionite-citrate-bicarbonate) method of Jackson (1958) as modified by Taylor & Crowder (1983). A known fresh weight of root (0.1 to 5g) was placed in a 100 ml conical flask containing a combination of 0.3M sodium citrate ($Na_3C_6H_5O_7$. $2H_2O$), 1.0M sodium bicarbonate ($NaHCO_3$) and sodium dithionite ($Na_2S_2O_4$). Relative amounts added were dependent upon the weight of root used (Table 2.1)

Fresh weight of root (g)	**Sodium bicarbonate (ml)**	**Sodium citrate (ml)**	**Sodium dithionite (g)**
0 -1	2.5	20	1.5
1-2	5	40	3
2-3	7.5	60	4.5
3+	10	80	6

Table 2.1 Quantities of chemicals used in the DCB extraction technique.

The flasks were covered and placed on an orbital shaker for 3 hrs at room temperature. After this period the resulting solution was filtered (Whatman No. 1 paper) into 100 ml volumetric flasks. The roots and conical flasks were rinsed thoroughly and the rinsings added to the volumetrics before making up to volume (100 ml) with UHP water. These were stored at 4°C until analysis. The remaining root was dried to a constant weight at 40°C and prepared for acid digestion.

In order to calculate the amount of metals in the plaque as mg kg^{-1} dry weight an equivalent section of root was taken from the same plant, weighed and then dried at 40°C until constant weight was reached. The roots were then re-weighed and the original fresh weight divided by the dry weight in order to give a conversion factor. This was carried out for each individual plant to ensure accuracy.

Acid Digestion

For small plant samples the most suitable extraction procedure is that of acid digestion. Approximately 0.3g of oven-dry plant material was weighed into 15 ml metal-free centrifuge tubes and the weight recorded. 5 ml of 30% HNO_3 was added to each and the tubes covered and placed in a heating block at 90°C overnight (8 hrs). The tubes were removed, allowed to cool and made up to volume (15 ml) with UHP water and stored at 4°C until analysis.

2.1.4 Preparation of plant roots for Scanning Electron Microscopy (SEM)

In order to achieve an accurate representation of the root sections as they are harvested it is necessary to preserve the root sections at the earliest opportunity. To achieve this the roots were cut into 1 mm sections with a clean razor blade and then put into acetone over a molecular sieve in an aluminium block cooled with liquid nitrogen. This was placed into a freezer (-80°C) for 2 weeks, after which the temperature was slowly raised to room temperature. The sections were transferred to a drying vessel and the acetone replaced by liquid carbon dioxide, the temperature was raised until the carbon dioxide was vented as a gas which left the samples in a dried state. Each section was then mounted upon a SEM stub and coated in carbon.

The roots were examined using a Jeol Scanning Microscope. The SEM is fitted with an Electron dispersive X-ray spectrometer for the chemical analysis of samples.

2.2 General methodologies for soil analysis

2.2.1 Aqua Regia Digests

The analysis of 'total' metal content of soils requires a strong extractant to solubilise metals from all soil fractions. A suitable method for this is aqua regia digestion. Approximately 3g of ground oven-dried soil was weighed into a Kjeldhal digestion tube. To this was added 23 ml of concentrated HCl and 7 ml of concentrated HNO_3 together with a few drops of a surfactant (dodecane). The samples were agitated and left overnight, a condenser was then placed on each tube and allowed to reflux for 2hrs on a heating block at 80°C. The tubes were allowed to cool and the resulting digest was filtered through a wetted filter paper (Whatman No 1) into a 100 ml volumetric flask. 1 ml of 10% KCl was added as an ionisation suppressant. The volume was made up to 100 ml with repeated UHP washings of the tube and filter paper and the resulting solution stored at 4°C until analysis.

2.3 General methodologies for porewater analysis

2.3.1 Extraction of pore water from soil

A number of methods have been used to extract pore waters from soils and sediments. These include squeezing which involves the use of gas pressure or direct pressure (Reeburgh 1967), centrifugation and *in situ* dialysis. However, no standardised technique has been found and therefore measurements are operationally defined. *In situ* sampling has the advantage of eliminating the need for handling and transporting the samples outside their normal environment, however this is an expensive and time-consuming method and was inappropriate for the sampling regime undertaken. When extracting samples *ex situ* one of the major sources of error is the oxidation of anoxic porewaters which has been shown to lower the concentration of iron and other elements (Bray *et al.* 1973; Troup *et al.* 1974; Loder *et al.* 1978; Lyons *et al.* 1979). However, acidification of samples allows them to be exposed to the air without chemical losses (Loder *et al.* 1978; Bufflap & Allen 1995). In addition, changes in temperature may also affect the

chemistry of porewater samples (Mangelsdorf *et al.* 1969; Bischoff *et al.* 1970; Fanning & Pilson 1971), however Troup *et al.* (1974) found that temperature variations did not effect on iron concentration in porewaters from anoxic sediments. It is therefore advised that samples should be stored at a low temperature similar to the field environment prior to, and following extraction. Due to the nature of the sediment involved, centrifugation was found to be unsuitable. In addition, the large number of samples prevented the use of gas filtration therefore in order to extract pore waters a new technique was designed which involved the use of direct pressure, giving approximately 10 ml of sample which was sufficient for the chemical analysis.

The soil sample was placed in a 50 ml plastic syringe and pressure applied until the porewater was extracted into an enclosed sample bottle. To remove particulate matter which can interfere with analytical procedures and can alter trace metal concentration, the resulting solution was filtered through a 0.2μm membrane filter (Whatman cellulose nitrate) into capped 15 ml tubes and made up to volume (10 ml) if necessary. Due to the acidic nature of the sampling sites it was necessary to acidify the samples with only 1-2 drops of concentrated HNO_3 and oxygen was excluded to the maximum extent possible at all stages to prevent any potential oxidation effects. This was achieved through the use of sealed containers and minimum exposure of the waters to the laboratory atmosphere. The samples were then frozen until analysis.

2.4 Analytical determinations of metals, anions and phosphorus

2.4.1 Control of Contamination

In experiments involving chemical determination of metals, particularly when dealing with trace levels, it is of vital importance to prevent contamination of samples and equipment. All experimental equipment such as glassware and sample bottles were washed in Decon for a minimum of 2 hrs, acid soaked overnight (>12 hrs) in 10% HCl and rinsed three times with UHP.

Elgastat UHP water (distilled, filtered, deionised, organic carbon removed and deionised again) with a purity of >18 MΩ cm^{-1} conductivity was used for all analytical solutions and all solution cultures.

All chemicals used were high purity Fisher 'AR' or BDH 'Analar' grade.

In any digestion technique it is necessary to include sufficient 'blanks' and standards to control for background contamination. One blank was prepared for every 15 samples, with a minimum of 4 per set of analyses. The mean of these blanks was then subtracted from sample readings. In addition, a minimum of 3 analytical standards were included in any plant digests. The standard used was hay powder (BCR reference material No. 129) which allowed for detection of error in analytical procedures.

2.4.2 Analytical procedures for metals

Atomic Adsorption Spectrometry

Concentrations of metals in all digests, extracts and surface water samples were measured by Atomic Absorption Spectrophotometry (AAS) on a Perkin-Elmer M2100 instrument. The standards were matrix matched to the sample. DCB solutions have been reported to give considerable interference with the adsorption of the Fe wavelength, Taylor & Crowder 1983; Taylor *et al.* 1984). However, when tested against known standards only limited interference was detected for all wavelengths used and diluted samples virtually eliminated any interference. DCB did, however, contain high concentrations of zinc and therefore any results had to be interpreted with care. During analysis an analytical quality control (AQC) or standard was inserted every 5 samples to ensure accuracy and precision of the instrument was maintained. Minimum detection limits for a selection of the metals present are shown in Table 2.2.

	Al	**Ca**	**Cu**	**Fe**	**Pb**	**Mg**	**Zn**
Minimum Detection Limit (mg l^{-1})	0.08	0.04	0.014	0.009	0.009	0.007	0.004

Table 2.2. Minimum detection limits for AAS analysis of metals.

Samples with values less than the detection limit are denoted as ND in text and tables.

Inductively-coupled plasma mass spectrometry

Concentrations of metals in all porewater extracts were analysed using an Inductively-coupled plasma-emission spectrometer (ICP-OES) on a Spectroflame M120 Bench-top Spectrometer. All standards were matrix matched to the samples. Calibration was achieved using 4 multiple standards at 5, 15, 25 and 200 mg l^{-1}. During analysis a standard was inserted every 10 samples to ensure that accuracy and precision of the instrument was maintained. Minimum detection limits for the elements analysed are shown in Table 2.3.

	Al	Ca	Cu	Fe	K	Mg	Mn	Na	P	Pb	S	Zn
Minimum Detection Limit (mg l^{-1})	0.003	0.003	0.003	0.003	0.01	0.003	0.003	0.01	0.01	0.015	0.01	0.003

Table 2.3. Minimum detection limits for ICP analysis of elements.

2.4.3 Analytical procedure for phosphorus

Phosphorus levels in all extracts and digests were measured using a Tecator 5012 flow injection analysis system. Samples were injected into a carrier stream and merged with a second carrier in order to avoid matrix effects. The combined stream was then mixed with an acidic ammonium molybdate solution (appendix B) to form heteropoly molybdophosphoric acid. This was then reduced to phosphomolybdenum blue using acidic stannous chloride (appendix C). The resulting blue colour was measured at 720 nm in a calibrated Tecator 5042 detector (Tecator Application Notes AN 60/83 & ASN 60-02/83). A 100 ppm P stock solution was prepared using anhydrous potassium dihydrogen orthophosphate (KH_2PO_4) which was used to make a series of standards. One standard was analysed for every 10 samples to maintain accuracy and precision.

2.4.4 Analytical procedure for anions

Concentrations of sulphate and chloride were determined using a Dionex 2000I, ion chromatograph system linked to a Trio integrating computer. Samples are pumped through a separator column made from an inert polystyrene/divinyl benzene cross linked resin where sample ions compete for cationic sites. Due to their larger ionic charge and ionic size chloride ions elute before the sulphate ions. The detection system is a conductivity detector with chemical suppression (2.8 mM sulphuric acid) thereby giving increased sensitivity. This measures the electrical conductivity of the ionic solution which is recorded.

Minimum detection limits of both sulphate and chloride are 0.1 mg/L. Experimental samples with values of less than the detection limit are denoted by ND in tables and text.

2.4.5 Minimum analytical detection limits

The detection limit of analytical equipment is defined as the minimum solution concentration that can be measured confidently above the background levels. For the Perkin-Elmer M2100 AAS the sample detection limit is the concentration of element giving a signal that is twice the magnitude of the baseline, and the sensitivity is the solution level of element required to produce a signal of 1% adsorption. Results that are measured between the detection limit and the sensitivity level can be interpreted as containing that element but at a trace level. This may be quantified on a more sensitive instrument. The Tecator 5042 detection limit of 0.05 mg l^{-1} is quoted for standard conditions, however the detection limit can be much lower but the time require to achieve this is impractical for large numbers of samples.

3. Metal cycling and distribution in wetland mesocosms.

3.1 Introduction

A variety of plant species have been used extensively in recent years within constructed wetlands designed to remove contaminants including heavy metals from polluted waters (e.g. Dodds-Smith *et al.* 1995; Scholes *et al.* 1998). However, the efficiency of such wetlands varies greatly and the inconsistency in wetland design and plant species used could be partly responsible. A number of investigations have found that plant species may vary in their ability to accumulate a number of metals and other contaminants. Gersberg *et al.* (1986) reported that *Scirpus validus* and *P. australis* were superior to *T. latifolia* in the removal of ammonia from water. Most studies have identified *T. latifolia* as generally being more efficient at taking up metals including Zn, Pb, Cr, Cd and Mn than *Sphagnum* (Wieder *et al.* 1990), *P. australis* (Mungur *et al.* 1997; Scholes *et al.* 1998) and *Scirpus robustus* (Meiorin 1989). Stark *et al.* (1995) did not find any difference between the metal accumulation of wetlands planted with *T. latifolia* and those with *Juncus effusus* L. or *Leersia oryzoides* L., (although the latter species are not normally associated with constructed wetlands). A number of explanations have been proposed to explain this apparent variation in metal uptake between species, including differences in the extent of root zone development (Gersberg *et al.* 1986), differences in the retention time of the contaminant (Wieder *et al.* 1990) and differences in the ability of plant species to sequester metals from the surrounding environment (Peverly *et al.* 1995). None of these explanations are completely satisfactory however and cannot be applied to all the species investigated.

The formation of iron plaque deposits upon the roots of wetland plants, particularly those growing in contaminated environments has been well documented in the literature (e.g Snowden & Wheeler 1995; Sundby *et al.* 1998). The presence of such plaques may affect the growth and development of plants in a number of ways. Firstly they may prevent or reduce the uptake of potentially phytotoxic metals into the plant tissues through their adsorption onto or co-precipitation with the iron oxyhydroxide, thereby allowing normal growth to continue in stressed environments. This may also

act to remove metals from contaminated waters. Secondly, they may inhibit growth by immobilising nutrients onto the plaque, again via adsorption and co-precipitation (Howeler 1973; Gambrell & Patrick 1978). These nutrients may however, be remobilised by the plant in times when nutrient supply is limiting though this has not been proved (Begg *et al.* 1994). The rate and extent of plaque formation therefore may affect the ability of plants to grow in contaminated environments where they are vital for trapping sediment, acting as a focus for microbial growth and possibly for actively removing pollutants from waters (Brix 1994). The loss of oxygen from plant roots is thought to be the main control on the development of iron oxyhydroxide plaques and this can vary greatly with plant species (Table 3.1).

Species	**O_2 release (mg h^{-1} /plant)**
T. latifolia (Cattail)	0.52
Acorus calamus (Sweet Flag Iris)	0.31
Iris pseudacorus (Yellow Flag Iris)	0.19
P. australis (Common Reed)	0.1

Table 3.1. R.O.L. of a range of wetland species (Stottmeister *et al.* 1998).

The variability between species may result in differences in their growth and the performance of wetlands in which they are used, but due to an absence of definitive information on iron plaques and the accumulation of metals within wetland systems, the extent to which this variability is important remains unclear.

In addition to differences between species, plaque development and uptake of metals may vary with season. Environmental factors which change with season have been found to influence the amount of both Fe and Mn on roots of *P. australis* in field situations (St Cyr & Crowder 1989, 1990). These include flooding and the amount of iron-bound-to-carbonates in the soil. Accumulation of plaque may also vary with the growth of *T. latifolia* (Crowder & Macfie 1986). It has been suggested that micro-organisms are also involved in the production of iron oxyhydroxides on roots (St Cyr

et al. 1993), and these would be subject to seasonal growth with a dormant period during the winter thereby affecting the extent of plaque formation.

Other plant characteristics also vary with time of year including P concentration and O_2 release (Fiala 1976; Gries & Garbe 1989; Gries *et al.* 1990; Van der Werff 1991) which could potentially affect metal uptake.

Few studies have addressed these major questions and there has been no attempt to relate plant development during the year to metal removal in wetlands. This is essential if the design of constructed wetlands is to be perfected for a variety of situations. The aims of the work reported in this chapter therefore were to:-

- Compare the uptake of metals in different wetland species
- Determine the relative importance of different metal sinks in wetland systems
- Assess the importance of season on the uptake and distribution of metals in wetlands.

3.2 Materials and methods

The majority of previous studies on metal uptake in wetland systems utilised either natural wetlands (e.g. Beining & Otte 1996; Keller *et al.* 1998), constructed wetlands (e.g. Stillings *et al.* 1988; Lan *et al.* 1992; Peverly *et al.* 1995; Sobolewski 1996; Scholes *et al.* 1998) or large wetland systems specifically designed for the study (e.g. Mandi *et al.* 1996). However, the use of natural systems does not allow the control of metal inputs or of the plant species present. Due to limited resources it was not possible to construct large experimental systems for this study. However, mesocosms have been successfully used to investigate processes in wetland systems (e.g. Wieder *et al.* 1990; Stark *et al.* 1995a,b, 1996; Mungur *et al.* 1997) and these are advantageous as they allow control of inputs and outputs of the system and resemble natural wetlands more closely than smaller, laboratory based systems.

Each wetland mesocosm in the present study was formed in a plastic bucket (capacity 30 l) which had a small hole drilled in the side to allow for drainage every week. A total of 30 plastic containers was used which allowed for 3 replicates of 5 treatments

each of which were repeated for summer and winter growth. Every bucket was filled to within 5 cm of the brim with a standard peat mixture (Table 3.2).

	Al	Cu	Fe	Mn	Zn
Concentration ($mg\ kg^{-1}$)	48.6 ±2.43	0.30 ±.06	298.75 ±14.39	2.62 ±.08	1.11 ±.26

Table 3.2. Concentration of selected heavy metals in the peat used in the mesocosm study. Means ± SE, n=3.

The buckets were then placed in an external experimental garden which allowed for natural conditions including day-length and temperature fluctuations, but to prevent contamination with rainwater and dust all tubs were covered with a sheet of black polythene secured with a length of rubber tubing. Each bucket was flooded with tap water and left for 24 hrs. At the end of this period each of the tubs was allocated to one of the following planting regimes:- *Phragmites australis; Typha latifolia;* a mixed community of *P.australis, T. latifolia* and *Iris pseudacorus;* bare soil and bare soil mixed with straw. Ten plants of each species obtained from a commercial grower were used in each tub where appropriate. In the mixed community this consisted of 3 plants each of *I. pseudacorus* and *T. latifolia* and 4 plants of *P. australis.* The plants chosen are species widely used in constructed wetlands both in the U.K. and the U.S.A. The straw treatment was set up to investigate whether the active root system was important in metal removal in the systems or whether it was simply the presence of organic material.

The tubs were then re-flooded and left for 100 days until new growth was observed. The pH of the water supply was then reduced to 3.5 through the addition of 0.1M H_2SO_4 and the tubs left for a further 30 days. During this period the water was changed every 7 days to prevent stagnation.

In order to induce plaque formation on plant roots prior to metal treatment, iron was added in the form of ammonium ferrous sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$) at a concentration of 100 mg/L in 5L of water. The tubs were then left for 7 days before artificial acid mine drainage (Table 3.3) supply was started.

	Concentration (mg l^{-1})	Compound used
Al	50	$Al_2(SO_4)_3$. $16H_2O$
Cu	10	$CuSO_4.5H_2O$
Mn	10	$MnSO_4$. $4H_2O$
Zn	30	$ZnSO_4$. $7H_2O$

Table 3.3. Composition of artificial AMD.

The composition of the mine drainage was based upon the concentrations of metals found in the output from a copper mine at Parys Mountain, Anglesey (Chapter 4). Solutions were replaced every 7 days in order to maintain supply of metals as in a natural system. After 50d growth in the AMD the buckets allocated to the summer harvest were removed and the plants harvested. Each plant was isolated from the bucket, rinsed well in UHP, divided into roots and shoots and digested according to the methods outlined in Chapter 2.1.3. The soil within each bucket was then mixed well and 5 sub-samples were taken of approximately 100g each, placed in clean plastic bags and stored at 4°C until digestion (Chapter 2.2.1). This sampling allowed for variations in metal distribution within the soil system. 5 further sub-samples were taken and the porewaters extracted from these according to Chapter 2.3.1

In order to allow direct comparisons of metal distribution and concentrations within the systems no further AMD was added to the buckets containing the winter harvest. Instead, tap water reduced to pH 3.5 was added to each tub weekly if there was evidence of drying out. This was continued for a further 120d, at the end of which the buckets were harvested as outlined above.

Statistical Analysis

Due to difficulties in harvesting, it was not possible to obtain absolute levels for metals in each section of the mesocosms and therefore all results are reported as concentrations. The effects of vegetation treatment and season on the distribution of

metals within the wetland mesocosms were examined using two-way ANOVA followed by a multiple comparison Tukey test. For the mixed treatments average concentrations from the different species were taken. A second analysis by two-way ANOVA examined the effect of plant species and season on metal uptake within these mixed systems.

Those data sets that failed the requirements of ANOVA (Zar 1996) were logarithmically transformed, usually either $\log_e$ or $\log_{10}$.

Specifics of the statistical results are tabulated in appendix D.

3.3 Results

3.3.1 Iron

Shoots

There was a significant effect of vegetation (ANOVA: $F=26.7$, $p<0.001$) and an interaction between vegetation and season ($F=5.0$, $p<0.05$) on Fe concentration in shoots.

Fe concentration was higher in straw than *T. latifolia, P. australis* or mixed culture shoots in summer, and higher in straw than *P. australis* and mixed culture shoots in winter. No significant difference was found between Fe shoot concentrations in *T. latifolia, P. australis* and mixed cultures in summer. Fe concentration in shoots was higher in the *T. latifolia* than *P. australis* and mixed cultures in winter.

There was no significant difference between season for any of the planting regimes.

Within the mixed culture system there was no significant effect of plant species, season or their interaction on Fe concentration in shoots.

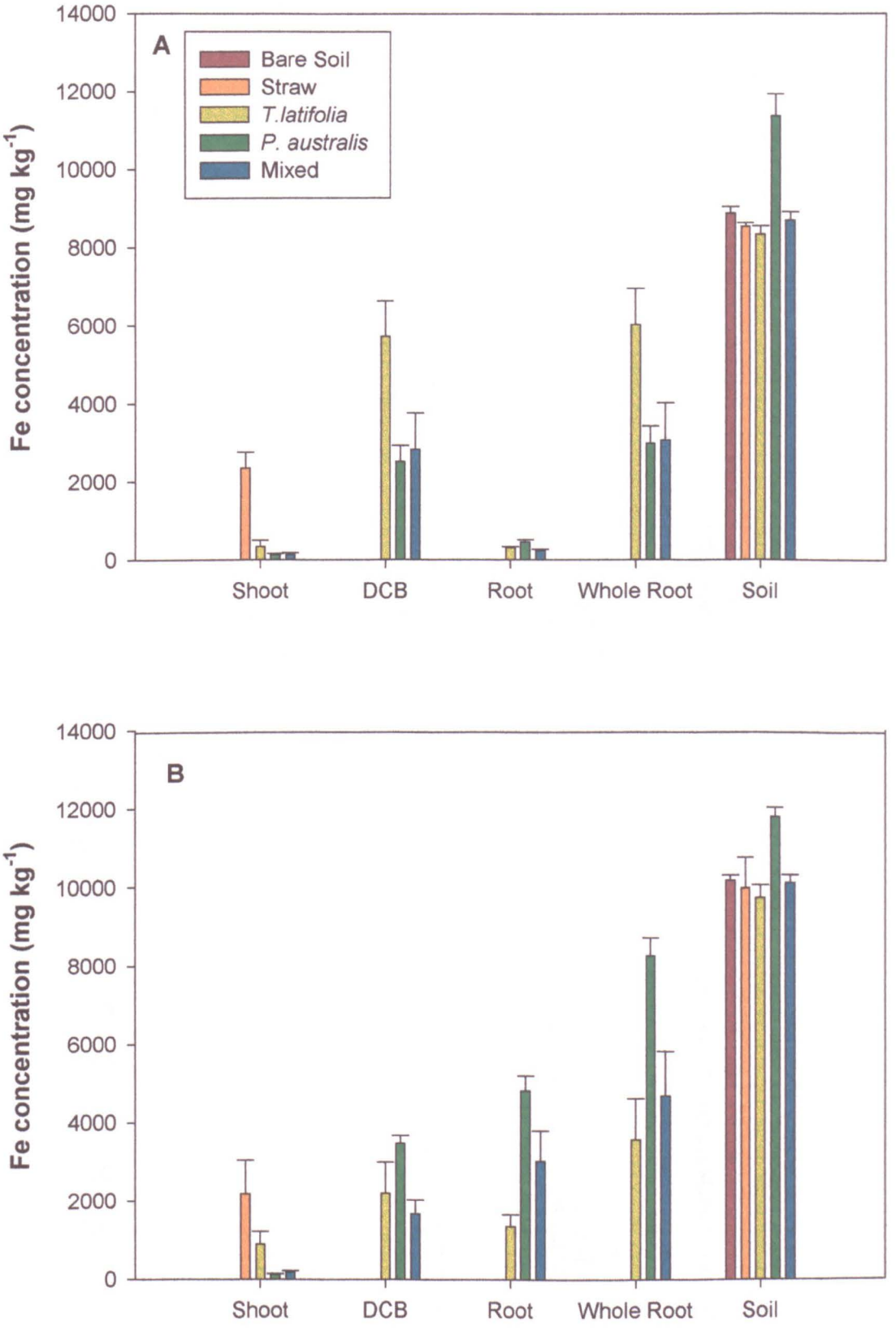


Figure 3.1. Fe concentration in components of single species wetland mesocosms in (A) Summer and (B) Winter.

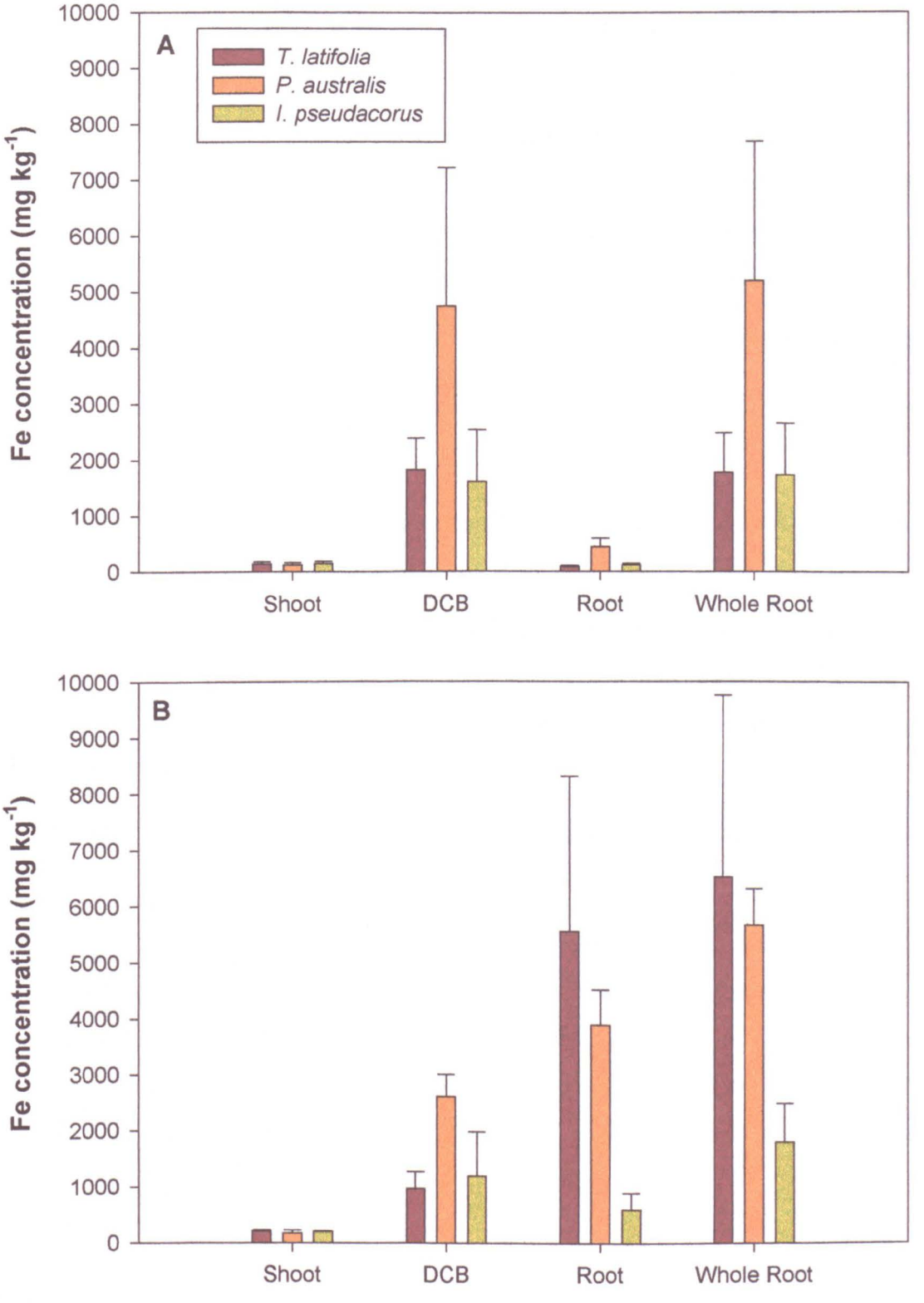


Figure 3.2. Fe concentration in components of mixed species wetland mesocosms in (A) Summer and (B) Winter.

DCB extract

There was a significant effect of season ($F=5.4$, $p<0.05$) and an interaction between vegetation and season ($F=5.7$, $p<0.05$) on Fe concentration in root DCB extracts.

There was no significant difference between planting regimes in the winter. However, Fe concentration was higher in *T. latifolia* in summer than *P. australis* and mixed cultures. In *P. australis* cultures Fe concentration was greater in winter than summer. In *T. latifolia* cultures Fe concentration was greater in summer than winter.

Within the mixed system there was no significant effect of plant species, season or their interaction on Fe concentration in DCB extracts.

Roots

There was a significant effect of vegetation ($F=14.1$, $p=0.001$), season ($F=239.8$, $p<0.001$) and the interaction between these treatments ($F=6.1$, $p<0.05$).

Significantly lower concentrations of Fe were found in *T. latifolia* than *P. australis* or mixed treatments in winter. In summer significantly higher concentrations of Fe were found in *P. australis* treatments than mixed, but this was not significantly greater than *T. latifolia* treatments.

Overall, Fe concentration was greater in all vegetation treatments in winter than summer.

Within the mixed system there was a significant effect of plant species ($F=13.0$, $p=0.001$), season ($F=87.5$, $p<0.001$) and the interaction between treatments ($F=8.0$, $p<0.01$) on Fe concentration in roots. In summer there was no significant difference between plant species. In winter, lower concentrations of Fe were recorded in *I. pseudacorus* than the other species. In all species, Fe concentrations were higher in winter than in summer.

Whole Roots

There was a significant effect of an interaction between vegetation and season ($F=9.9$, $p<0.01$) on Fe concentration in whole roots.

Higher concentrations of Fe were found in winter than summer in *P. australis* treatments, but no significant difference was found between seasons for the remaining vegetation regimes.

Within the mixed treatment there was no significant effect of plant species, season or the interaction between treatments on Fe concentrations in whole roots.

Soil

There was a significant effect of vegetation ($F=4.6$, $p<0.01$), season ($F=28.7$, $p<0.001$) and the interaction between these treatments ($F=13.1$, $p<0.001$).

In both summer and winter there was a greater concentration of Fe in *P. australis* treatments than other treatments.

Higher Fe concentrations were recorded in soils from bare soil, straw, *T. latifolia,* and mixed treatments in winter than in summer. No significant effect of season was found for the *P. australis* treatment.

Porewater

There was a significant effect of vegetation ($F=7.0$, $p=0.001$), and an interaction between vegetation and season ($F=2.9$, $p<0.05$) on Fe concentration in porewater.

In winter there was a significantly higher concentration of Fe in the porewater from the straw treatment than the *P. australis* and mixed cultures. There was no significant difference between the bare soil, *T. latifolia, P. australis* and mixed treatments. In summer however, Fe concentration was higher in the *T. latifolia* culture than the bare soil, *P. australis* or mixed treatments. The Fe concentration was significantly higher in the *T. latifolia* in the summer than winter.

Summary

- Fe concentrations were higher in DCB extracts, roots and whole roots of *P. australis* in winter than summer

- Fe concentrations were lower in DCB extract of *T. latifolia* in winter than summer
- Fe concentrations were greater in shoots of straw than all other vegetation types.

3.3.2 Manganese

Shoots

There was a significant effect of vegetation ($F=23.3$, $p<0.001$) and season ($F=5.9$, $p<0.05$) on Mn concentration in shoots.

A greater concentration of Mn was found in shoots of the *T. latifolia* treatment than the other planting regimes in both summer and winter.

Mn concentrations were higher in shoots from the *P. australis* treatment in summer than winter. There was no significant difference between seasons for the remaining treatments.

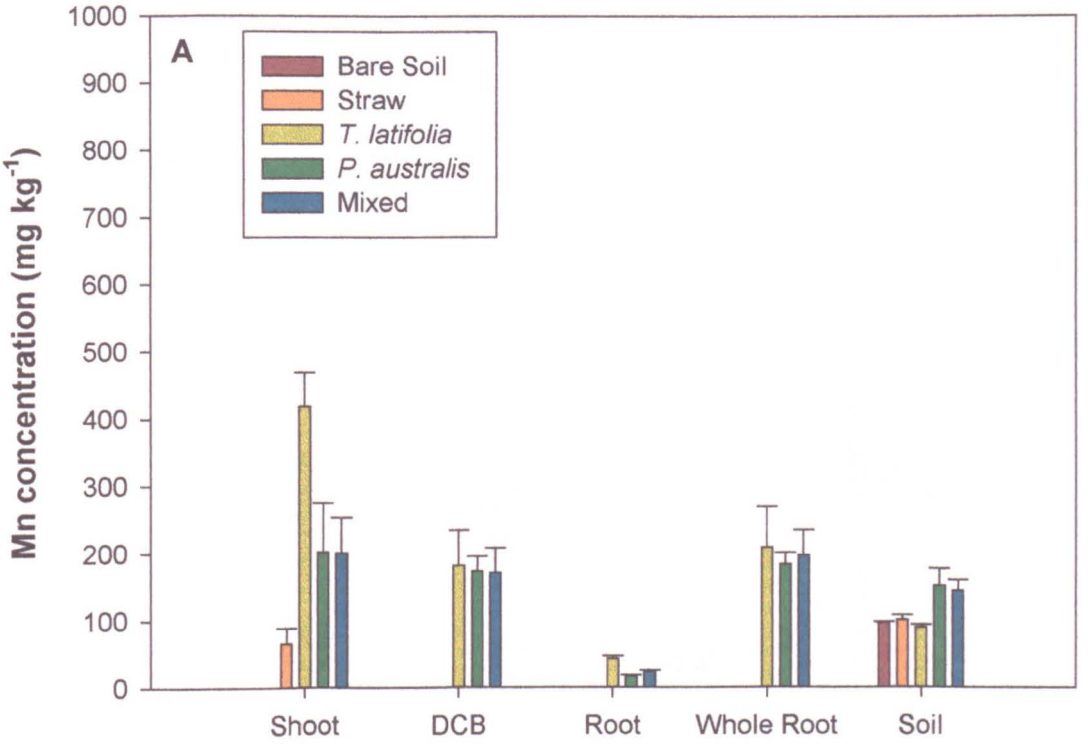
Within the mixed system there was a significant effect of plant species ($F=15$, $p<0.001$) on Mn concentrations in shoots. In winter lower concentrations were found in *P. australis* than the other species.

DCB Extract

There was a significant effect of season ($F=12.7$, $p<0.01$) on Mn concentration in the DCB extract.

Higher values of Mn were found in the DCB extract of roots from summer treatments when compared with winter treatments.

Within the mixed system there was a significant effect of season ($F=6.8$, $p<0.05$) on Mn concentrations in DCB extracts. Concentrations were lower in winter than summer.



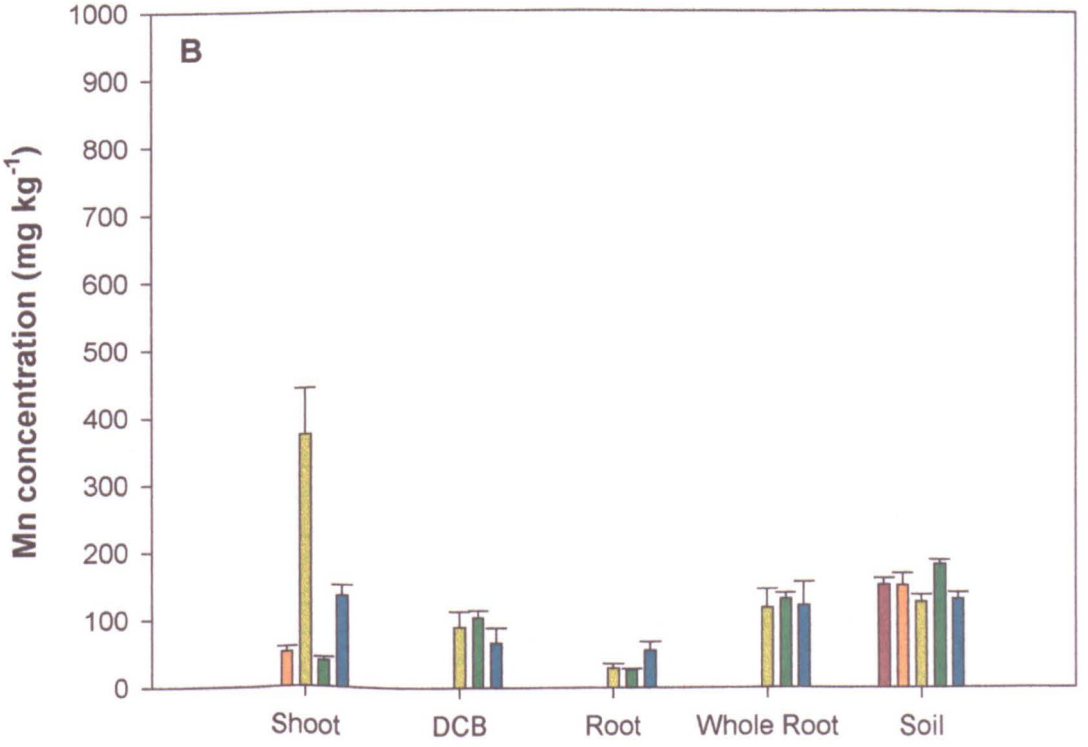


Figure 3.3. Mn concentration in components of single species wetland mesocosms in (A) Summer and (B) Winter.

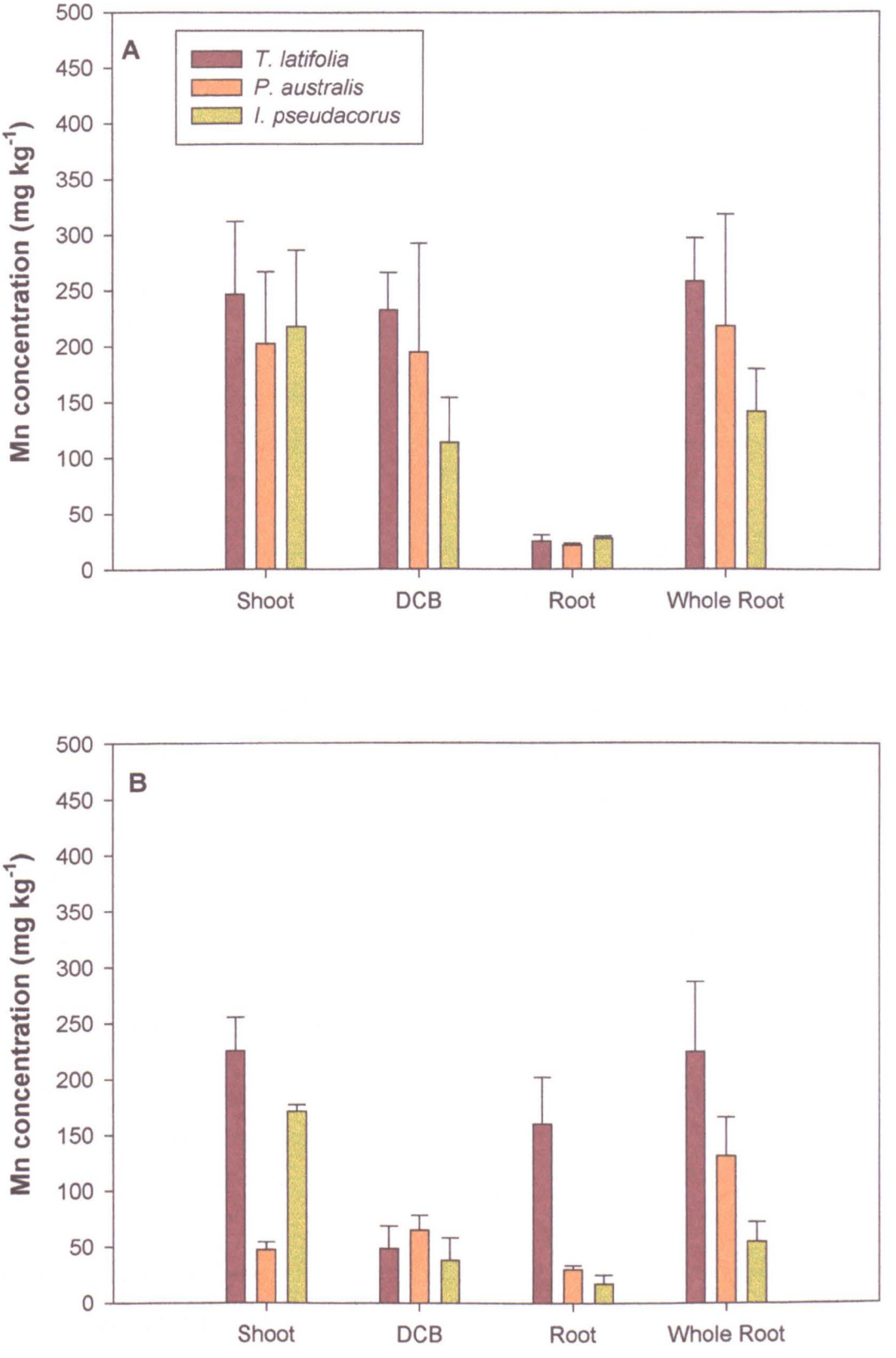


Figure 3.4. Mn concentration in components of mixed species wetlands in (A) Summer and (B) Winter.

Roots

There was a significant effect of vegetation ($F=6.3$, $p<0.05$) and the interaction of vegetation and season ($F=7.5$, $p<0.01$) on Mn concentration in roots.

Greater concentrations of Mn were found in roots from the mixed treatment than the *P. australis* treatment in winter. In summer Mn concentrations were higher in roots from the *T. latifolia* treatment than the *P. australis* treatment.

Mn concentrations were higher in summer than winter in *T. latifolia* and mixed treatments. No significant difference was found between the seasons for the *P. australis* data.

Within the mixed system there was a significant effect of plant species ($F=11.3$, $p<0.01$), season ($F=5.8$, $p<0.05$) and the interaction between the treatments ($F=13.3$, $p=0.001$) on Mn concentrations in roots.

In summer there was no significant difference between plant species, however in winter concentrations were higher in *T. latifolia* than the other species. In addition, Mn concentrations were higher in *T. latifolia* in winter than summer.

Whole Roots

There was a significant effect of season ($F=6.6$, $p<0.05$) on Mn concentration. Overall greater concentrations of Mn were found in whole roots from the summer treatments than those from winter.

Within the mixed treatment there was no significant effect of plant species, season or the interaction between treatments on Mn concentration in whole roots.

Soil

There was a significant effect of season ($F=13.3$, $p<0.01$) and the interaction between vegetation and season ($F=7.4$, $p=0.001$).

In winter, higher Mn concentrations were found in soils from the *P. australis* treatment than those from *T. latifolia* and mixed regimes. In summer greater Mn values were recorded in soils from the *P. australis* and mixed treatments than the remaining planting regimes.

Significantly higher concentrations of Mn were found in bare soil and straw treatments in summer than winter. No significant effect of season was found for the remaining treatments.

Porewater

There was a significant effect of vegetation ($F=12.2$, $p<0.001$), season ($F=12.2$, $p<0.001$) and the interaction between these treatments ($F=2.9$, $p<0.05$).

Fe concentration was higher in the porewater from the bare soil treatment in summer than any other treatment, and this was also higher than the bare soil treatment in winter.

Summary

- Mn concentrations were higher in DCB extracts, roots and whole roots of *T. latifolia* in summer than winter.
- Mn concentrations were greater in shoots and roots of *T. latifolia* than other vegetation treatments in the summer.
- Mn concentrations were higher in soils of *P. australis* than *T. latifolia* treatments in both summer and winter

3.3.3 Zinc

Shoots

There was a significant effect of vegetation ($F=13.4$, $p<0.001$) on Zn concentration in shoots.

In winter there were greater concentrations of Zn in shoots from the straw and *T. latifolia* treatments than those from mixed cultures. In addition Zn values were higher in shoots from the straw treatment than *P. australis* treatment in both winter and summer.

Within the mixed treatment there was no significant effect of plant species, season or the interaction between treatments on Zn concentration in shoots.

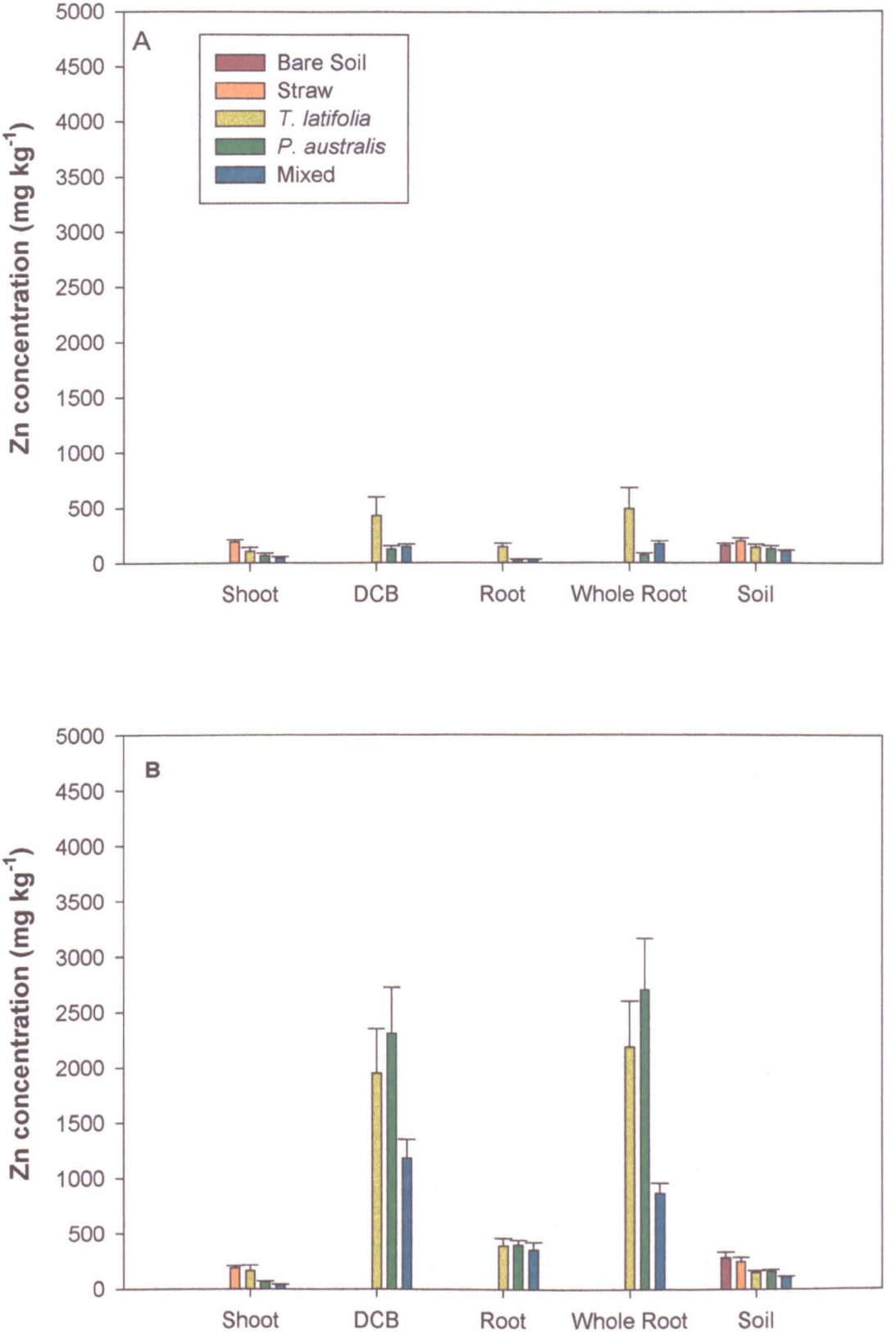


Figure 3.5. Zn concentration in components of single species wetland mesocosms in (A) Summer and (B) Winter.

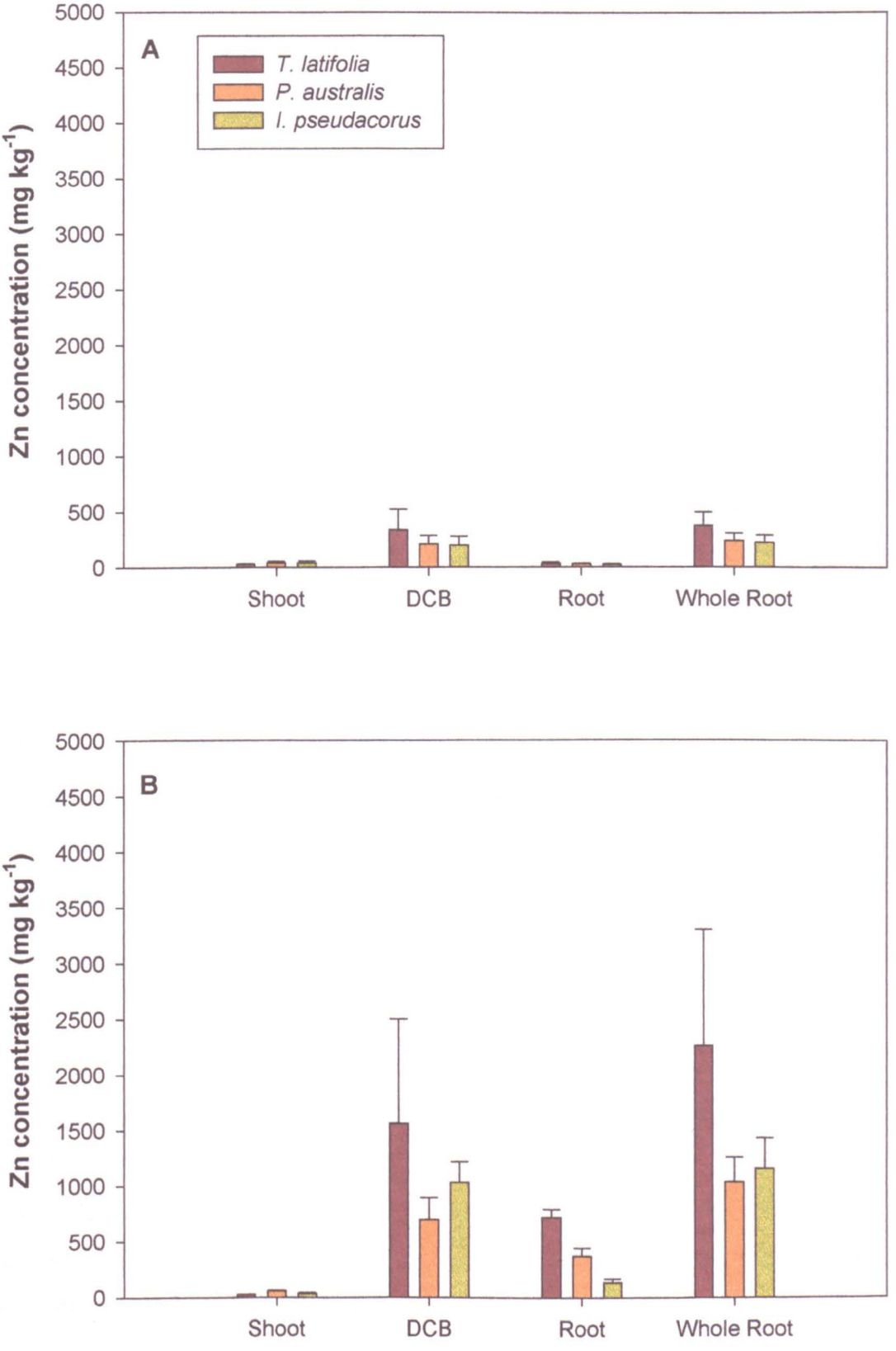


Figure 3.6. Zn concentration in components of mixed species wetland mesocosms in (A) Summer and (B) WInter.

DCB extracts

There was a significant effect of vegetation ($F=5$, $p<0.05$), season ($F=134$, $p<0.001$) and the interaction between these treatments ($F=4.4$, $p<0.05$).

No significant difference was found between the vegetation treatments in winter. In summer greater concentrations of Zn were found in DCB extracts from roots of the *T. latifolia* treatment.

Overall, higher Zn values were recorded in root DCB extracts from winter than summer treatments.

Within the mixed system there was a significant effect of season ($F=10.3$, $p<0.05$) on Zn concentration in DCB extracts. Higher concentrations of Zn were found in DCB extracts in the winter than summer.

Roots

There was a significant effect of vegetation ($F=15.9$, $p<0.001$), season ($F=236.2$, $p<0.001$) and the interaction between these treatments ($F=14.5$, $p=0.001$).

In winter there was no significant effect of planting regime on Zn concentration. In summer greater concentrations were found in roots from the *T. latifolia* treatment than *P. australis* and mixed cultures.

Overall, higher concentrations were found in roots from winter than summer treatments.

Within the mixed system there was a significant effect of plant species ($F=25.9$, $p<0.001$), season ($F=131.4$, $p<0.001$) and the interaction between treatments ($F=24.5$, $p<0.001$) on Zn concentration in roots. In summer there was no significant difference between plant species, however in winter higher concentration of Zn were found in *T. latifolia* than the other species. In addition *P. australis* had higher concentrations of Zn than *I. pseudacorus.*

Zn concentrations in the roots of *T. latifolia* and *P. australis* were higher in winter than summer.

Whole Roots

There was a significant effect of vegetation ($F= 11.4$, $p<0.01$), season ($F=175.2$, $p<0.001$) and the interaction between the treatments ($F=15$, $p=0.001$).

In the mixed treatment, lower concentrations of Zn were found in winter than the *P. australis* and *T. latifolia* treatments. In summer greater concentrations were recorded in whole roots from the *T. latifolia* treatment than the *P. australis* and mixed cultures. In addition there were higher Zn concentrations in the mixed culture than the *P. australis* treatment.

Overall, higher concentrations were found in the winter than summer treatments.

Within the mixed system there was a significant effect of season ($F=20.6$, $p<0.001$) on Zn concentration in whole roots. In all species concentrations were higher in winter than summer.

Soil

There was a significant effect of vegetation ($F=6.3$, $p<0.01$) and season ($F=5.4$, $p<0.05$) on Zn concentrations in soil.

In winter higher Zn values were recorded in soils from the bare soil treatment than the mixed treatment, but these were not significantly higher than the other treatments. Values were also significantly higher in soils from the straw treatment than the mixed.

No significant effect of planting regime was found in summer.

Porewater

No significant effect of vegetation, season or the interaction between the treatments was found.

Summary

- Zn concentrations were higher in DCB extracts, roots and whole roots in winter than summer.

3.3.4 Aluminium

Shoots

There was a significant effect of vegetation ($F=28$, $p<0.001$) and season ($F=4.7$, $p<0.05$) on Al concentration in shoots.

Significantly higher concentrations were found in shoots from the straw treatment in winter. In addition, higher concentrations were found in the *T. latifolia* than *P. australis* treatment.

In summer higher concentrations were also found in the straw treatment, but this was not significant when measured against the *T. latifolia* treatment. There were also higher values recorded for the *T. latifolia* treatment when compared with the *P. australis* treatment.

There were significantly higher concentrations of Al in straw shoots in winter than summer, but no significant effect of season on the remaining vegetation treatments.

Within the mixed system there was no significant effect of plant species, season or the interaction between treatments on Al concentrations in shoots.

DCB extracts

There was a significant effect of season ($F=47$, $p<0.001$) on Al concentrations.

Higher concentrations of Al were found in the DCB extracts of roots from *T. latifolia* and mixed treatments in summer than winter. No significant effect of season was found for the *P australis* treatment.

Within the mixed system there was a significant effect of season ($F=35.7$, $p<0.001$) on Al concentrations in DCB extracts. Concentrations in winter were higher in *T. latifolia* and *P. australis* than in summer.

Roots

There was a significant effect of vegetation ($F=23.6$, $p<0.001$), season ($F=41.2$, $p<0.001$) and the interaction between these treatments ($F=6.0$, $p<0.05$).

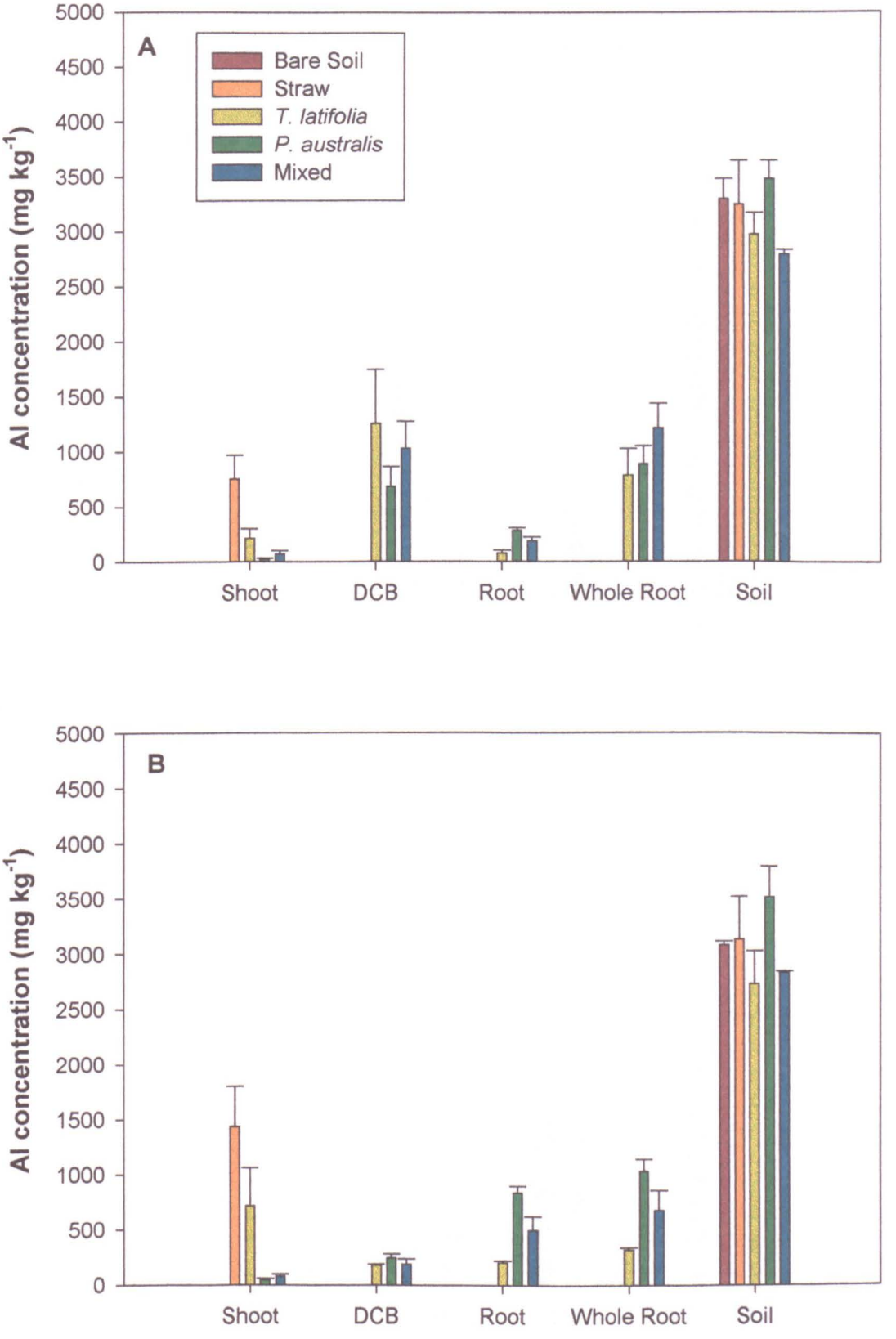


Figure 3.7. Al concentration in components of single species wetland mesososms in (A) Summer and (B) Winter.

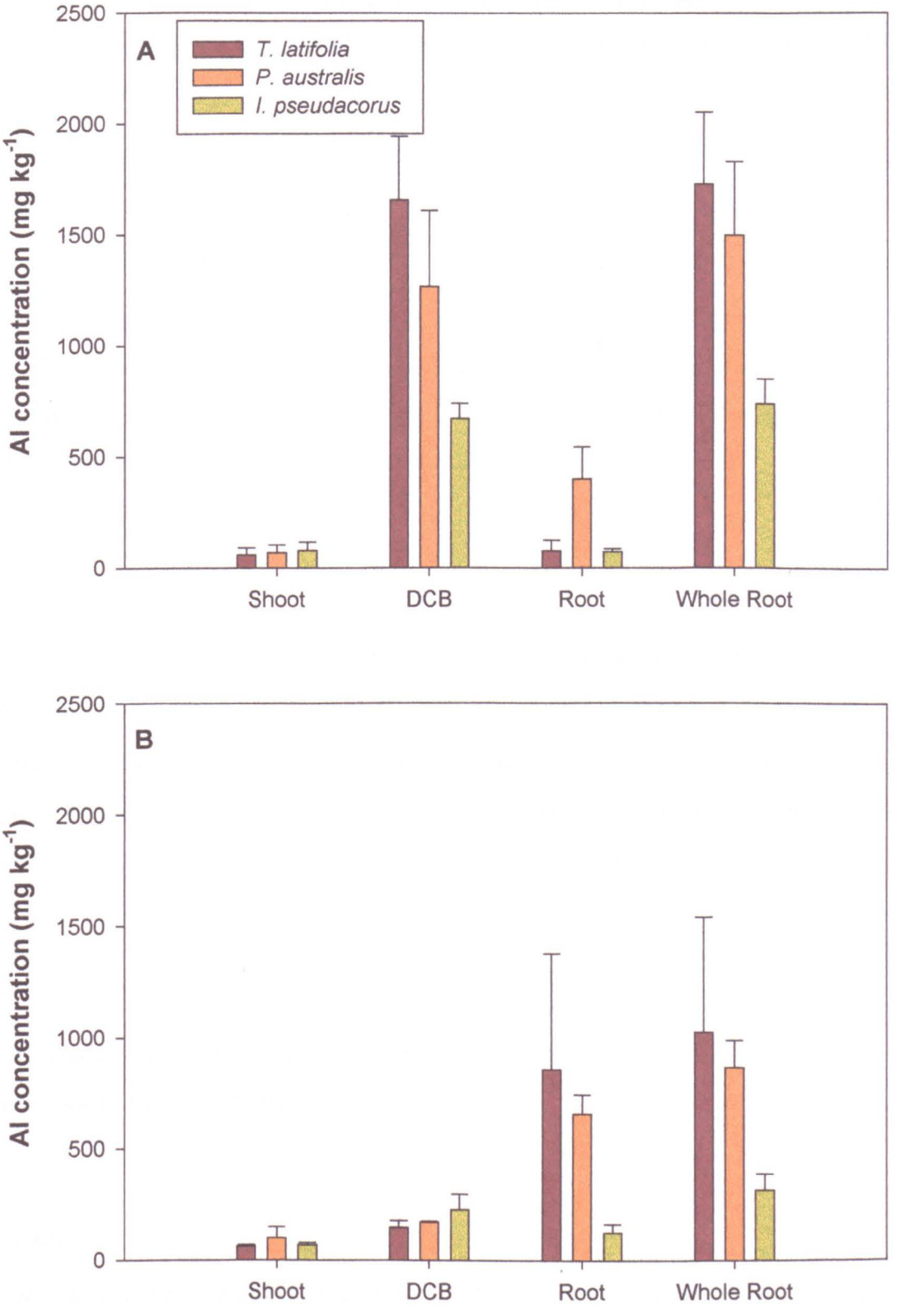


Figure 3.8. Al concentration in components of mixed species wetland mesocosms in (A) Summer and (B) Winter.

In winter higher values of Al were found in roots from the *P. australis* treatment. Greater concentrations in the mixed treatment were also recorded when compared to the *T. latifolia* culture.

No significant effect of vegetation was found in summer.

There were higher concentrations of Al in roots from *P. australis* and mixed cultures in winter than summer.

Within the mixed system there was a significant effect of plant species ($F=4.8$, $p<0.05$) and season ($F=8.1$, $p<0.05$) on Al concentrations in roots. In winter there were significantly lower concentrations in *I. pseudacorus* than the other species. In addition concentrations were higher in winter than summer.

Whole Roots

There was no significant effect of vegetation, season or the interaction between these treatments on Al concentrations.

Within the mixed system there was a significant effect of plant species ($F=6.6$, $p<0.05$) and season ($F=7.2$, $p<0.05$) on Al concentration in whole roots. There were lower concentrations in the whole roots of *I. pseudacorus* than *T. latifolia*.

Soil

There was no significant effect of vegetation, season or the interaction between these treatments on Al concentrations.

Porewater

There was a significant effect of season on Al concentration in porewater ($F=13.3$, $p<0.01$).

Concentrations were generally higher in winter when compared with summer.

Summary

- No significant effect of season was found for DCB extracts or whole roots of *P. australis.*
- Al concentrations were higher in roots of *P. australis* than the other vegetation treatments in winter.

3.3.5 Copper

Shoots

There was a significant effect of vegetation ($F=12.2$, $p<0.001$) and season ($F=5.2$, $p<0.05$) on Cu concentrations.

Greater concentrations of Cu were found in shoots from the straw treatment than the other treatments in both summer and winter. This was not significant for the *P. australis* treatment in summer.

Within the mixed system there was a significant effect of plant species ($F=8.2$, $p<0.01$) on Cu concentrations in shoots. *P. australis* had significantly higher concentrations of Cu than *I. pseudacorus.*

DCB extracts

There was a significant effect of season ($F=19.5$, $p=0.001$) on Cu concentrations.

In *T. latifolia* and *P. australis* treatments there were higher values of Cu in summer than winter.

Within the mixed system there was no significant effect of plant species, season or the interaction between the treatments on Cu concentrations in DCB extracts. It appeared that values were lower in *P. australis* than the other species in summer but the large standard error resulted in a non-significance.

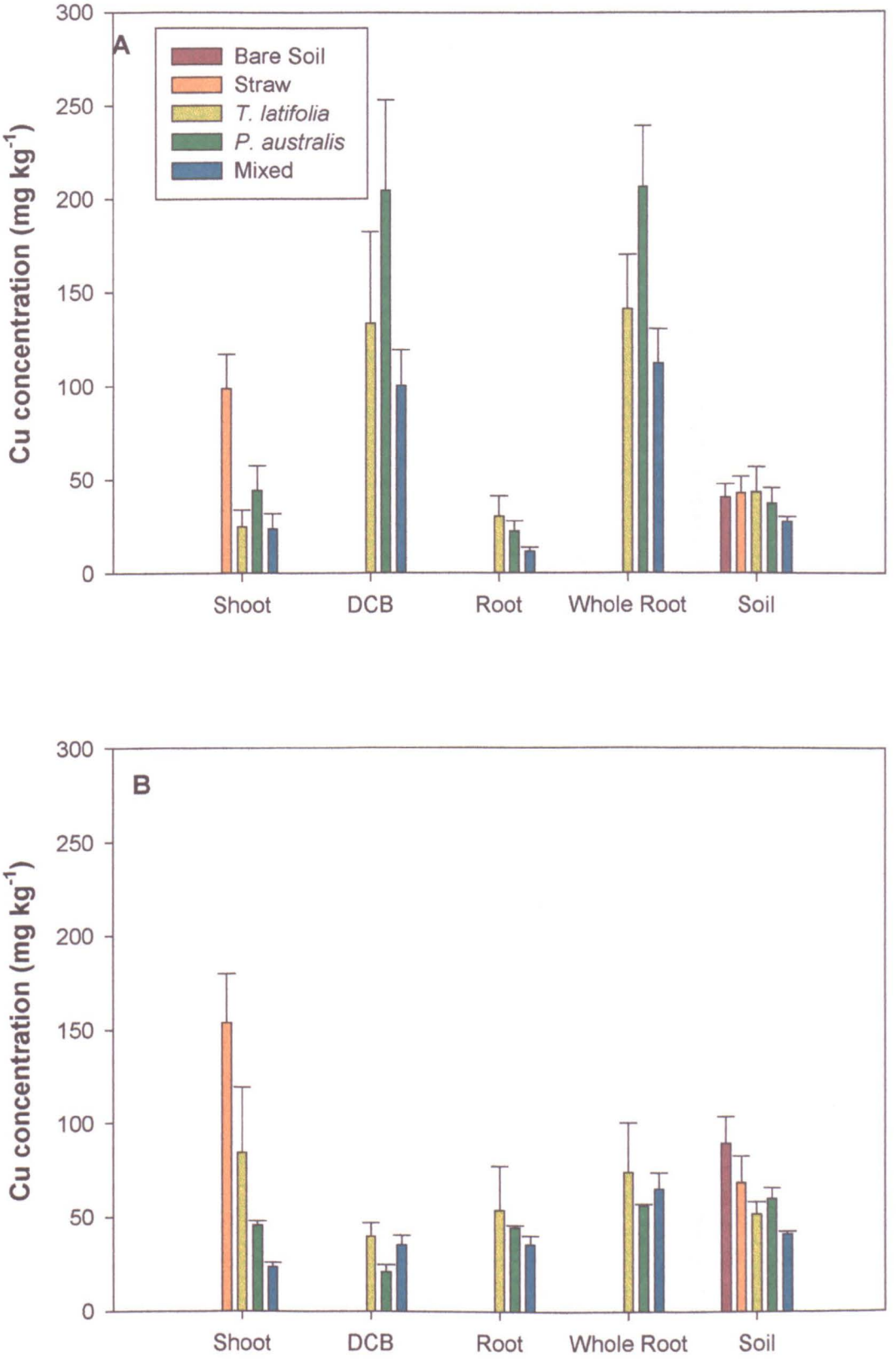


Figure 3.9. Cu concentration in components of single species wetland mesocosms in (A) Summer and (B) Winter.

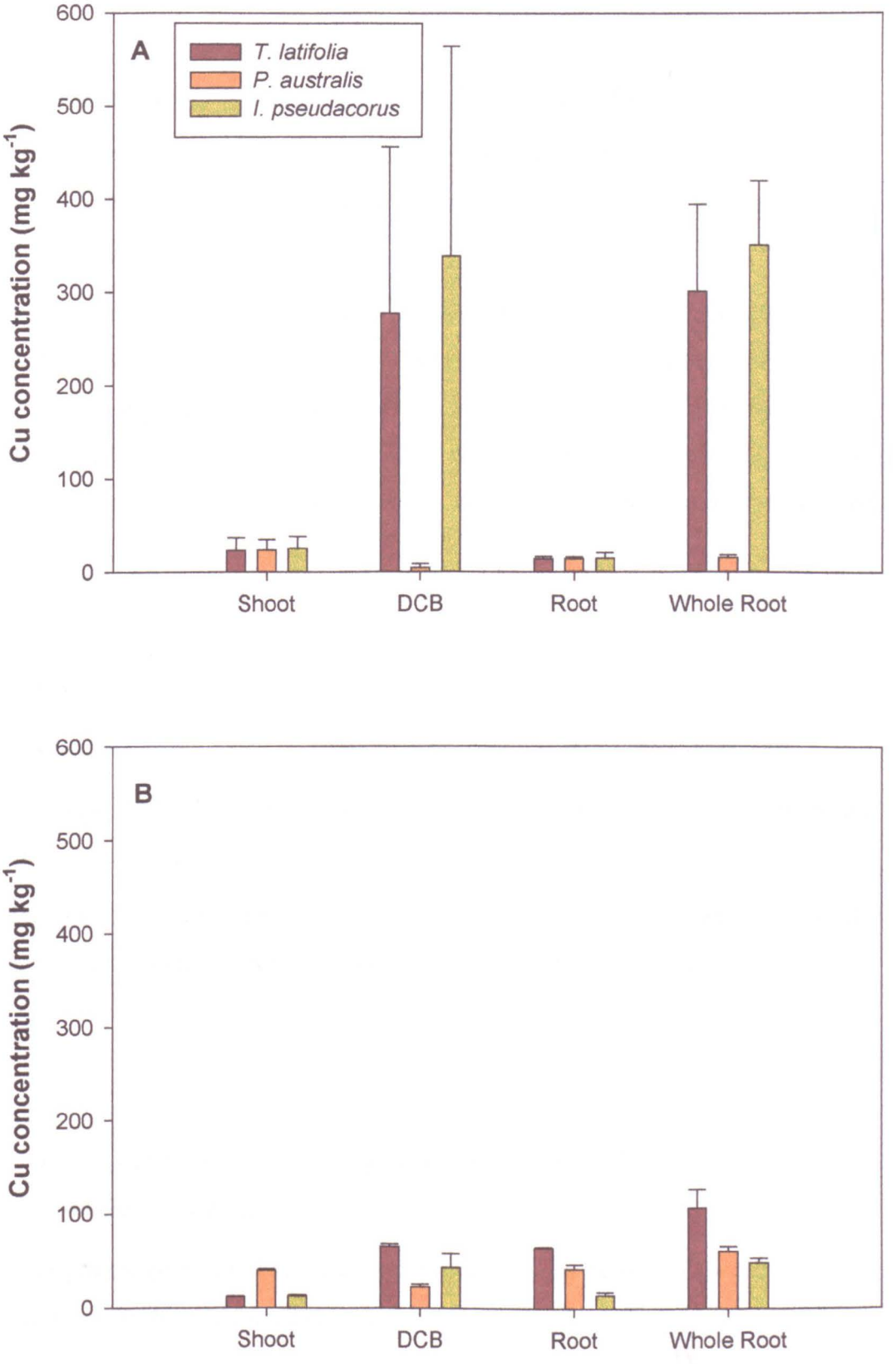


Figure 3.10. Cu concentration in components of mixed species wetland mesocosms in (A) Summer and (B) Winter.

Roots

There was a significant effect of season ($F=13.9$, $p<0.01$) on Cu concentrations.

Greater concentrations of Cu were recorded in the mixed treatment in winter when compared with summer. No significant effect of season was found for the remaining vegetation treatments.

Within the mixed system there was a significant effect of plant species ($F=17.6$, $p<0.001$), season ($F=56.1$, $p<0.001$) and the interaction between treatments ($F=19.1$, $p<0.001$) on Cu concentrations in roots. In the winter harvest higher concentrations were recorded in *T. latifolia* than the other two species and *P. australis* contained higher concentrations than *I. pseudacorus*. Both *P. australis* and *T. latifolia* had elevated concentrations in winter when compared to summer.

Whole Roots

There was a significant effect of season ($F=4.8$, $p<0.05$) on Cu concentrations.

Overall, significantly lower concentrations were found in whole roots from winter than summer treatments.

Within the mixed system there was no significant effect of plant species, season or the interaction between the treatments on Cu concentrations in whole roots.

Soil

There was a significant effect of vegetation ($F=3.0$, $p<0.05$) and season ($F=16.2$, $p=0.001$) on Cu concentrations.

In winter greater concentrations of Cu were found in soils from the bare soil treatment than the *P. australis* and mixed cultures.

No significant effect of vegetation treatment was found in the summer.

Soils from the bare soil treatments had lower Cu concentrations in summer than winter.

Porewater

There was no significant effect of vegetation, season or the interaction between these treatments on Cu concentrations.

Summary

- Cu concentrations were higher in DCB extracts and whole roots of *T. latifolia* and *P. australis* treatments in summer than winter
- Cu concentrations were higher in shoots of straw treatment than the other vegetation treatments.

3.3.6 Phosphorus

Shoots

There was a significant effect of vegetation ($F=14.4$, $p<0.001$), season ($F=7.9$, $p<0.05$) and the interaction between these treatments ($F=3.6$, $p<0.05$) on P concentrations.

In winter there were greater concentrations of phosphate-P in shoots from the *P. australis* treatment.

In summer significantly lower values were found in shoots from the straw treatment.

Overall, there were higher P concentrations in summer than winter in the *T. latifolia* culture.

Within the mixed system there was a significant effect of plant species ($F=4.43$ $p<0.05$) and season ($F=17.42$ $p<0.01$) on P concentration in shoots. In winter there were higher concentrations in shoots of *I. pseudacorus* than the other species. Overall, concentrations were higher in summer than winter.

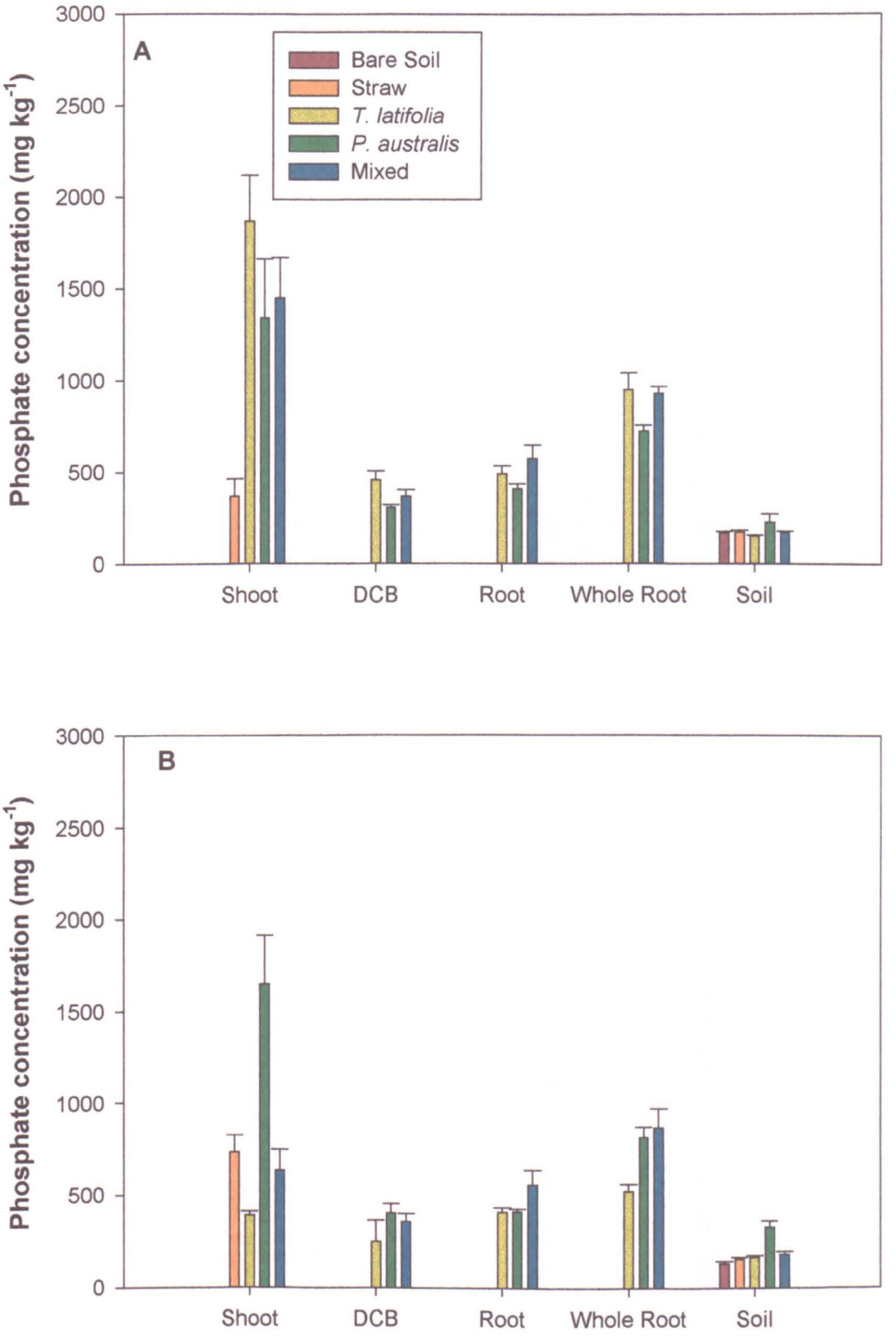


Figure 3.11. P concentration in components of single species wetlands in (A) Summer and (B) Winter.

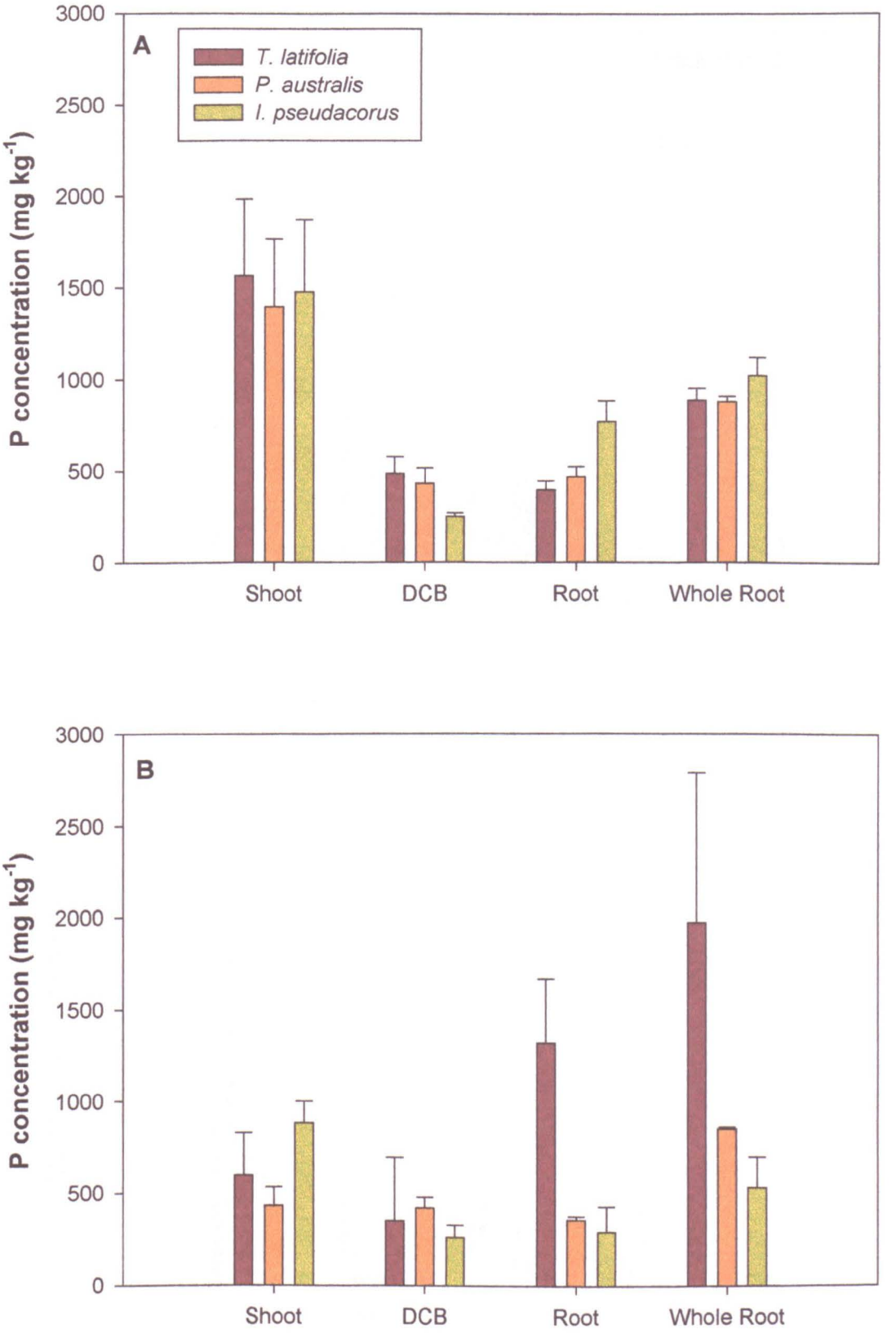


Figure 3.12. P concentration in components of mixed species wetland mesocosms in (A) Summer and (B) Winter.

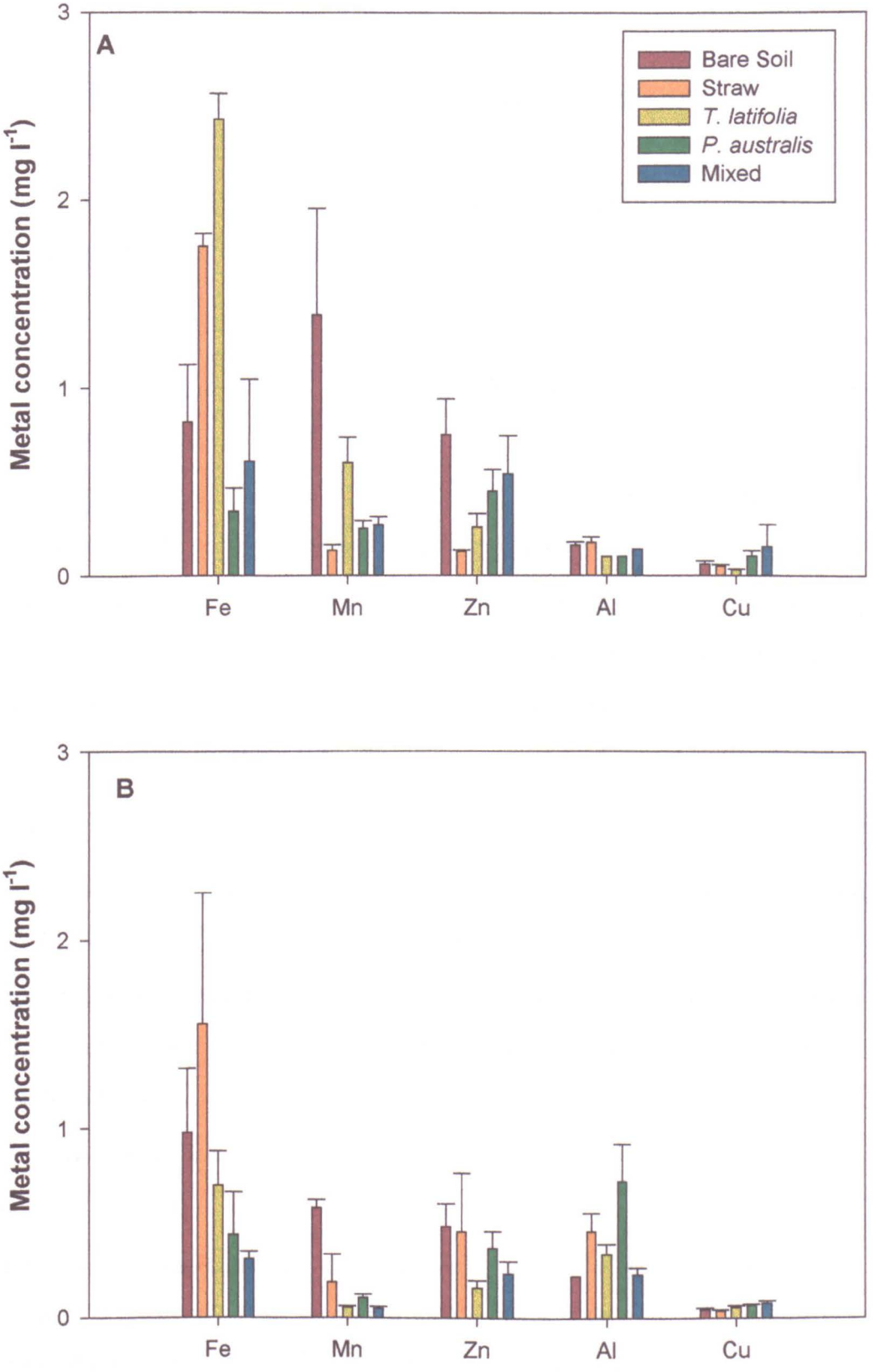


Figure 3.13. Metal concentration in porewaters from single species wetland mesocosms in (A) Summer and (B) Winter.

DCB extracts

There was no significant effect of vegetation, season or the interaction between these treatments on P concentrations.

Within the mixed system there was no significant effect of plant species, season or the interaction between these treatments on P concentrations.

Roots

There was a significant effect of vegetation ($F=5.9$, $p<0.05$) on phosphate-P concentrations.

Concentrations of P were higher in roots from the mixed treatment than the *P. australis* treatment. No other significant differences were found.

Within the mixed system there was a significant effect of the interaction between plant species and season ($F=12.4$, $p<0.001$) on P concentrations in roots. In winter there were significantly higher concentrations in *T. latifolia* than the other species and values were also higher in winter than summer.

Whole Roots

There was a significant effect of season ($F=5.5$, $p<0.05$) and the interaction between vegetation and season ($F=8.2$, $p<0.01$) on P concentrations.

In winter, P concentrations were lower in whole roots from the *T. latifolia* treatment in comparison with the *P. australis* treatment. No significant effect of vegetation was found in the summer.

Overall, there were higher P concentrations in summer than winter in the *T. latifolia* culture.

Within the mixed system there was a significant effect of plant species ($F=4.2$, $p<0.05$) and the interaction between plant species and season ($F=6.1$, $p<0.05$) on P concentrations in whole roots. The pattern of distribution was similar to roots with higher values recorded in *T. latifolia* than the other species in winter, and concentrations in *T. latifolia* were higher in winter than summer.

Soil

There was significant effect of vegetation ($F=5.7$, $p<0.01$) and the interaction between vegetation and season ($F=10.8$, $p<0.001$) on P concentrations.

There was significantly higher P values for soils from the *P. australis* treatments in both winter and summer when compared with the *T. latifolia* treatments.

Overall, there were higher P concentrations in winter than summer in the *P. australis* culture.

Summary

- No significant effect of vegetation or season was found for the DCB extracts.
- P concentrations were higher in shoots and whole roots of *P. australis* than *T. latifolia* in winter.

3.4 Discussion

Iron

In the present study it was found that Fe contents of the root DCB extracts (a measure of plaque development) were highest in summer for *T. latifolia.* No additional metals were added between the summer and winter harvest in order to allow direct comparisons, therefore the reduction in Fe content of the root plaques of *T. latifolia* suggests that Fe was remobilised in winter. R.O.L. may be an important factor in the formation iron oxides around the roots of wetland plants (Chapter 1). The release of oxygen should reach a maximum level when the growth of the plant is greatest, during spring and summer and thus the formation of iron plaques should also be at its highest level during this period. Crowder and Macfie (1986) reported greatest plaque accumulation on the roots of *T. latifolia* in June and July corresponding to the peak biomass production of the shoots which levelled off by late July and August. In addition, Gries *et al.* (1990) reported maximum accumulation of iron plaque on roots of *P. australis* in the month of July. As R.O.L. decreases in the winter this could allow the remobilisation of Fe from the root plaques which could either be released into the soil environment or taken up by the plant tissues.

The shoot and whole root data however, showed that Fe content of the whole roots actually decreased in the winter, and shoot content increased but only to a limited extent which does not account for the decrease of Fe content in the root plaques. In contrast Fe concentration in the soils from the *T. latifolia* treatments increased in the winter which suggested that there was some release of Fe back into the surrounding environment in the months of dormancy. However, this was not the case for either *P.australis* or mixed cultures, with similar Fe concentrations measured in the winter harvest. This is unexpected as *P. australis* has two main periods of growth, one in April and the other at the end of September (Fiala 1976) and therefore oxygen release would have been greater in the summer harvest resulting in a greater development of root plaque. However, oxygen transport through the dead culms of *P. australis* has been shown to be sufficient to keep the roots aerobic through the dormant period (Gries *et al.* 1990). This could therefore allow Fe plaques previously formed in the summer to remain throughout the winter and thus prevent release of Fe from the plaques into the surrounding environment as in the case of *T. latifolia.* The soil data supported this hypothesis with no significant difference between Fe concentrations in summer and winter.

In the mixed cultures there was a reduction in Fe concentration in all species in winter which suggests that there is some degree of interaction between the roots of the different plants. During the oxidation of iron, H^+ ions are released, therefore upon reduction of the iron plaques, H^+ ions should be consumed which could result in a raising of the pH of the local environment. This may lower the availability of iron for plaque formation and so iron plaque production could cease around the roots of the adjacent *P. australis.* There is a corresponding increase in root Fe concentration which suggests that there is some uptake of Fe into the roots in winter which could partly originate from mobilisation of the iron plaques in both species, either through chemical processes or the release of exudates from the roots.

These results suggest that iron oxide formation was primarily caused by oxygen release by roots. However, acid digests of straw gave high concentrations of Fe in both winter and summer indicating that Fe may be precipitated on to the surface of the straw. From a comparison of these with results from the surface of the roots it is evident that there was little difference between the straw and other planting regimes

except for *T. latifolia* in summer and *P. australis* in winter which had higher concentrations. It is possible that the iron precipitation on the straw surfaces was mediated by bacteria and may conceivably have formed a different iron oxide mineral from that on the root surfaces. This mineral may be more stable than the iron oxides on the root surfaces and therefore would not be subject to dissolution in the winter so preventing the release of iron back into the surrounding environment. However, Fe concentrations in the soil from the straw treatment were higher in the winter than the summer but the origin of this iron remains unclear.

Manganese

Mn has also been reported to form oxide plaques either in isolation or combination with Fe plaques (Bacha & Hossner 1977; St Cyr & Crowder 1987, 1990; Crowder & Coltman 1993; St Cyr & Campbell 1996). If R.O.L. is the key mechanism by which Mn plaques form then the pattern of Mn oxide precipitation should correlate with Fe precipitation. However, Mn was found to be higher in all the vegetation treatments in summer than winter. This suggested that in all plant treatments there was remobilisation of Mn during the winter months. Mn does not oxidise as easily as Fe and therefore, although flow of oxygen through *P. australis* during the winter was sufficient to retain the iron plaque, it may have been insufficient to allow the Mn plaque to remain. Shoot and whole root concentrations of Mn were lower in the winter than summer indicating that the Mn liberated during the winter months was not taken up by the plants. Neither was it retained by the soil as concentrations of Mn in soil did not differ in winter and summer for the planted treatments.

In addition, Mn oxide formation does not chemically occur below a pH of about 8.6, therefore under acidic conditions it is more likely to be microbially mediated. Microbial action will be higher in summer months and may cease in winter thereby potentially resulting in the remobilisation of Mn oxides into the surrounding environment.

Within mixed cultures Mn concentrations in shoots were higher in *T. latifolia* and *I. pseudacorus* relative to *P. australis* in winter. These results agree with those from the single species treatments where there is reduction in Mn content of the shoots of

P. australis in the winter, but no such reduction for the other species. There is no equivalent increase in the remaining metal sinks and therefore it is unclear where this Mn is sequestered.

Aluminium

It has been reported in the literature that Fe and Mn oxides may prevent or reduce the uptake of other metals due to their adsorption onto or co-precipitation with the Fe and Mn oxides (Otte *et al.* 1987; Greipsson & Crowder 1992; Greipsson 1994; Wang & Peverly 1996). It has already been suggested that during the winter months Fe and Mn may be released back into the environment due to dissolution of the plaques on *T. latifolia*. Any metals co-precipitated should therefore, also be liberated. Al concentration in the DCB extract agreed with this hypothesis as Al remained at a similar level in summer and winter for *P. australis* but was significantly lower in winter for *T. latifolia,* suggesting that Al was associated with the Fe. However, in the mixed cultures Al concentrations in the DCB extracts were lower for all species in winter than summer. Al oxides readily form in the sedimentary environment and it is possible that Al oxides may form around roots as a result of R.O.L. from the roots. During the winter months when R.O.L is lower the Al oxides could be remobilised into the surrounding environment as in the case of both Mn and Fe oxides.

Copper

Cu concentration was significantly lower in DCB extracts from winter roots for both *T. latifolia* and *P. australis* treatments. It is unclear where this Cu was sequestered because shoot, whole root and soil data did not show any increase in Cu concentration in the winter harvest.

When grown in a mixed culture *T. latifolia* accumulates a greater concentration of Cu in DCB, roots and whole roots in winter than *P. australis.* This suggests that when these species are present in the same environment the *T. latifolia* competes for uptake of nutrients and thus metals. This is surprising as *P. australis* in the mixed cultures produced a greater mass of roots which would have enabled the plants to exploit a larger volume of soil. It is inferred from this that *T. latifolia* has a greater ability to

take up and accumulate metals in its roots. However, when grown in isolation there is no significant difference in accumulation of these metals in the winter. Cu concentrations in the shoots of these plants did not differ significantly and were at a low level in all.

Zinc

Zn concentrations in DCB extracts were lower in all planting treatments in summer and a number of explanations for this pattern may be proposed. Due to loss of organic material including carbon in the winter months through die-back of shoots and roots, the relative concentration of metals could appear to be elevated owing to the method of calculation. However, it would be expected that the other metals would also show a similar increase in the winter months but this was not the case. Kirk & Bajita (1995) have shown that as iron oxidation proceeds this results in the acidification of the rhizosphere due to release of H^+ ions, and this could also increase the availability of other metals including Zn which may result in higher concentrations in the root. However, this is unlikely as the other metals did not show a similar increase in concentration and the increased Zn did not occur during the periods of maximum iron plaque development in all vegetation treatments. Although there was some dissolution of iron plaques on the roots of *T. latifolia* in winter, not all of the iron was released back into the environment and some plaque remained on the roots. The adsorption of Zn on to plaque has previously been reported (Ye *et al.* 1998a; Otte *et al.* 1989; St Cyr & Campbell 1996; Doyle & Otte 1997; Sundby *et al.* 1998). The adsorption capacity of the plaque for Zn may not be reached during the summer and therefore Zn may be expected to continue to bind to the remaining plaque during the winter months. Although some Zn would be released by the dissolution of the plaque this would be re-absorbed onto the remaining plaque coating. As a result a higher concentration of Zn would be found in winter for all species. The absence of a corresponding elevation of Cu and Al may be due to their lower affinity from iron oxides, however adsorption of Zn onto both iron oxides and sediment particles has been proved to be lower than that of Cu (Machemer & Wildeman 1992; Hamilton-Taylor *et al.* 1997; Lin & Chen 1998). Furthermore, the binding of Cu on amorphous

Fe oxides has been shown to be sufficiently strong to overcome the competition of other cations (Johnson 1986).

The cation exchange capacity of roots could also constitute a possible explanation for the increase in Zn in the winter. In the winter the roots of the wetland plants die off and this would result in sloughing off of the epidermal cells and disruption of the root surface. This could produce and increase the amount of cation exchange sites available to the Zn ions. This would allow Zn to adsorb to the root surface and therefore produce the increase in Zn concentration in the DCB extract. In addition, in the *T. latifolia* treatment dissolution of the plaques could expose more of the root surface and its adsorption sites so allowing Zn to adsorb onto the surface. This hypothesis would assume that Zn has a higher affinity for these cation exchange sites than the other metal cations as no corresponding increase in the concentration of these metals is recorded.

It has previously been suggested that the DCB extraction technique is harsh (Bacha & Hossner 1977; McLaughlin, Van Loon & Crowder 1985) and this could result in the removal of metals not only from the external surface of the root but also from within the cells. This would give an overestimation of the metal content of the DCB extract. The whole root data would therefore give a more accurate measure of metal concentration and this shows a similar increase in Zn concentration in the winter. However, this could be attributed to gradual accumulation of Zn in the roots of the plants as the metal is taken up from the surrounding environment. Concentration of Zn in the roots of *T. latifolia* and *P. australis* has previously been reported (Lan *et al.* 1992; Ye *et al.* 1997 c, 1998a,b,).

Within the mixed cultures Zn concentrations were higher in DCB, roots and whole roots of *T. latifolia* than *P. australis* showing a similar pattern to Cu. This again suggests that in mixed species cultures *T. latifolia* has a greater capability of accumulating metals in roots than *P. australis* and *I. pseudacorus.* When grown in isolation however there is no significant difference in the ability to accumulate Zn between *P. australis* and *T. latifolia* in winter.

The presence of iron phosphates have been reported in plaques (Snowden & Wheeler 1995) and therefore it would be expected that phosphate-P concentration would be elevated when iron plaque formation is at a maximum. However, there was no significant difference in DCB extract concentration between species or season which differs from the pattern seen for Fe. This suggests that in this system phosphate is not associated directly with the iron plaque.

3.5 Conclusions

The results demonstrated that within mixed cultures there is a significant difference between the uptake of metals with the order of *T. latifolia* > *P. australis* > *I. pseudacorus* for all metals except Cu where uptake is greater in *I. pseudacorus* than *P. australis* but only in the summer. However, in the separate vegetation treatments there is little difference between *P. australis* and *T. latifolia* in metal uptake but both in some cases are more efficient than the mixed cultures. From this it can be suggested that wetlands planted with single species are more efficient for metal removal and in particular *P. australis* takes up more metals. However, in wetlands planted with a number of species *T. latifolia* competes with *P. australis* and therefore has a greater potential for metal uptake.

In addition it was suggested that in single species cultures Fe could be remobilised during the winter months from the root plaques of *T. latifolia* therefore allowing the possibility of a release of contaminants back into the environment, whereas the movement of air through the dead culms of *P. australis* prevents this release of Fe. The formation of Al oxides on roots was also suggested and the patterns of re-release were similar to those of Fe. Therefore *P. australis* is more efficient in removing metals from the environment when planted in a monoculture and does not allow the re-release of metals back into the surrounding soil in the dormant period.

4. Metal removal processes in contaminated wetlands.

4.1 Introduction

Wetland soils are important in biogeochemical cycles because they form a major interface between the terrestrial and aquatic ecosystems. Within sediment and soil systems there is a tendency for chemical equilibrium to develop between the interstitial water and solid phase. However, this is not usually achieved due to diagenetic processes including precipitation, adsorption, sulphide formation, remobilisation and biological uptake, resulting in a chemically dynamic system. Porewater chemistry has been used in a number of studies to investigate the occurrence of a range of elements in such wetland systems, the majority of which have concentrated on the distribution of sulphur species (Boulegue *et al.* 1982; Luther *et al.* 1986; Canfield 1989; Smolders & Roelofs 1996; Bottrell & Novak 1997). Processes of sulphate reduction and pyrite formation have been shown to be extremely important, but in addition, plant growth may also affect the chemistry of these systems (Caçador *et al.* 1996; Templer *et al.* 1998). Plant roots can interact with the surrounding sediment and interstitial waters through the uptake of nutrients, cations and anions and the release of CO_2 and organic compounds. The release of oxygen and root exudates from wetland plants results in the formation of iron (oxy-) hydroxide precipitates on the root surfaces. It has been suggested that these root plaques may prevent the uptake of other potentially phytotoxic metals into plant tissues through adsorption and co-precipitation processes, which would result in the depletion of metals from the surrounding interstitial waters and their concentration in the iron plaques. Chapter 3 demonstrated that root processes, in particular the formation of root plaques could affect the uptake from, and release of heavy metals into the surrounding environment. In addition the enrichment of Cd, Cu, Pb and Zn has been reported in rhizoconcretions forming around the roots of *Spartina maritima* growing in salt marshes, which was accompanied by metal depletion in the surrounding sediments (Sundby *et al.* 1998). An equivalent investigation into the effect of plant growth and specifically the formation of iron plaques on the chemistry of interstitial waters has yet to be reported. This information would add to the

understanding of the chemistry of plaque formation and the relative importance of plant growth in the distribution and immobilisation of metals in contaminated wetland systems.

The specific aims of the work reported in this chapter were therefore:-

- to investigate the distribution of a range of elements in contaminated wetland systems
- to determine the extent of changes in distribution with seasonality
- to identify the processes that control this distribution

4.2 Materials and Methods

Two field sites were selected that had been receiving acid mine drainage for a number of years and that contained an established community of wetland vegetation including the Common Reed, *Phragmites australis*.

4.2.1 Site Descriptions

Parys Mountain

Parys Mountain (OS 450900) is situated 3 km south of Amlwch in north-east Anglesey, Wales forming a north-northeast trending ridge 2.5 km long and 150m above sea level (Figure 4.1). The area is sited upon a syncline with a core of Silurian shales overlying a volcanic sequence of rhyolitic tuffs, lavas and pyroclastics. Associated with the volcanics are a series of polymetallic sulphide minerals which occur in three geological settings:-

(a) Disseminated mineralisation hosted by siliceous sinter or 'quartz rock' occurring at the contact between the Parys shales (Ordovician) and Parys Mountain volcanics

(b) Disseminated or massive mineralisation hosted by shales and/or volcaniclastic sediments, again associated with the base of the main volcanic pile

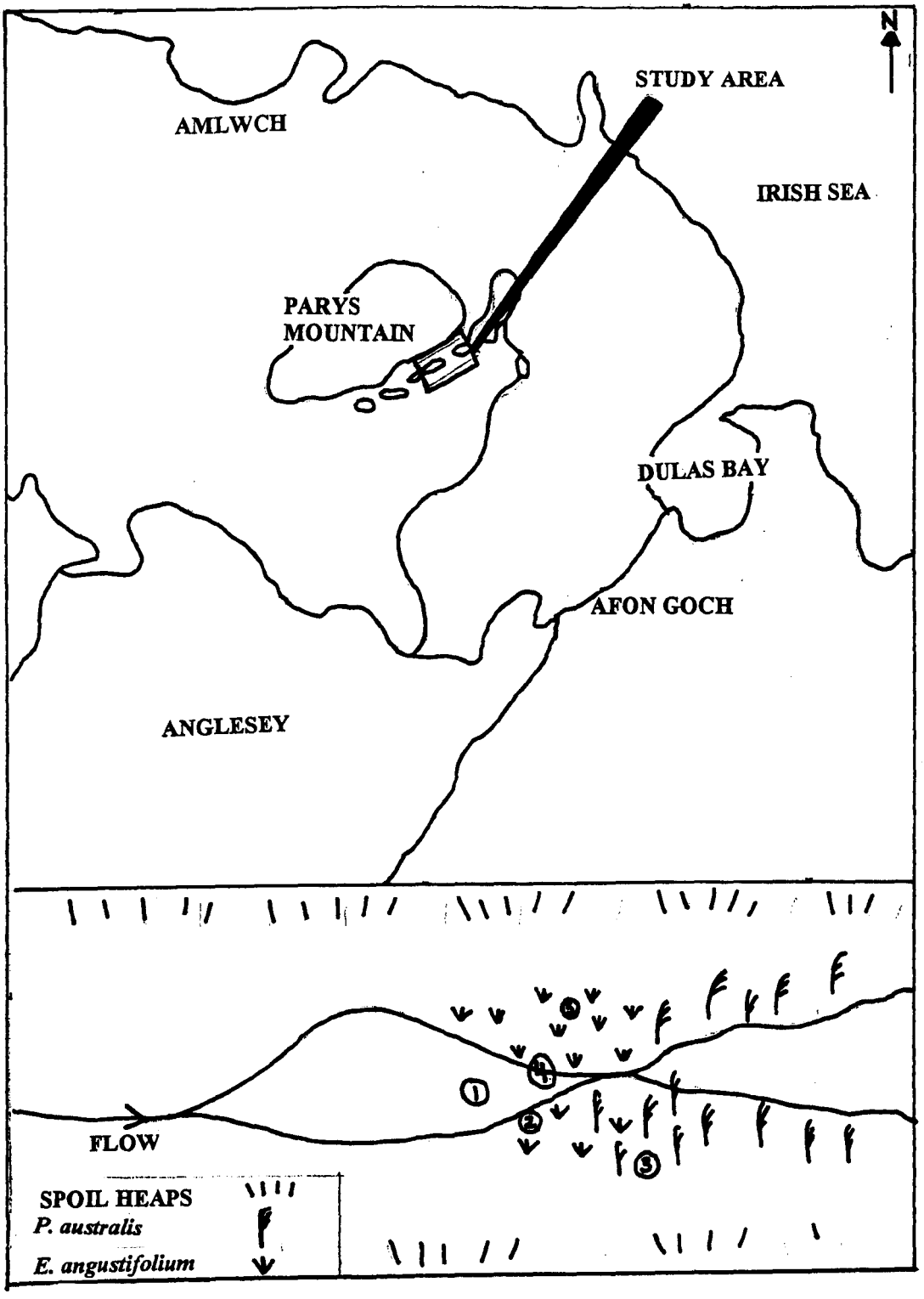


Figure 4.1.Location map of Parys Mountain.

(c) Massive or disseminated mineralisation hosted by volcaniclastic sediments and occurring approximately 80 m above the base of the volcanic sequence (Southwood 1982).

The ore mineral assemblages are of chalcopyrite ($CuFeS_2$), galena (PbS), sphalerite (ZnS) and pyrite (FeS). Trace element analyses also revealed anomalous concentrations of Cu, Zn, Pb, Ni, Co, Cr, Hg, Ba and Sr around the mineralised zones (Thanasuthipitak 1975).

The area has been the site of sporadic copper extraction from Roman times although no works appear to have been undertaken between the Roman period and the 18th century (Greenly 1919). During the late 18th and early 19th centuries it was Europe's premier copper mine delivering >3050 t/yr copper together with minor amounts of lead and zinc ore (Southwood 1984). Ore was initially mined from shafts but these collapsed and so forced opencast working, although further shafts were sunk during the 19th century. By 1920 all hard rock mining had ceased but precipitation pools were still in use. The pools were used to extract copper electrochemically from drainage waters through the addition of scrap iron. After the exchange of copper for iron the mud from the bottom of the pools was removed, dried and processed.

At present the area consists of the shafts, which have been capped, together with a large void containing a pool of acidic water (pH <3.0) with a smaller void to the north-east. With the exception of the precipitation pools the whole site is covered by mine spoil. Historical mining operations such as those of Parys Mountain tended to be very inefficient in terms of separation, and much of the ore is left within the tailings. In the case of Parys, 40% of the original ore still remains constituting a significant source of contamination. The exposure of the sulphide minerals to the air during mining results in the oxidation of sulphide minerals and the production of sulphuric acid. This in turn increases the mobility of metals which are abundant in abandoned mine sites, resulting in the production of acid mine drainage (Plates 4.1 & 4.2).

Plate 4.1. Mine spoil heaps at Parys Mountain.

Plate 4.2. Contaminated drainage at Parys Mountain.

Plate 4.3. Precipitation pools at Parys Mountain.

Plate 4.4. *P. australis* growth at Parys Mountain.

These extremely acidic waters drain the site through old settling ponds (Plate 4.3) and constitute the source of the southern Afon Goch. This stream drains a catchment of 35-40 km^2 and runs 11 km to its tidal limit where it enters the sheltered estuary of Dulas Bay. As the stream leaves the main spoil area a population of *Phragmites australis* has become established together with a smaller area of *Eriophorum angustifolium*. The drainage waters pass through this vegetation before flowing down towards the estuary (Plate 4.4).

Mam Tor

The area known as Mam Tor is situated at the head of the Hope Valley, near Castleton, North Derbyshire (Figure 4.2). The area is characterised by a still active landslide of the slump earthflow type (Varnes 1958). The Mam Tor landslide is one of many in the area which are usually located where Kinderscout Grit or Shale Grit overlie steep shale slopes. At Mam Tor the Mam Tor Beds and underlying Edale Shales are affected (Plate 4.5).

The Mam Tor Beds consist of a grey micaceous sandstone alternating with subsidiary siltstone and shale. These are typical distal turbidites. The sandstone units are typically more resistant to erosion than the interbedded shales and form ledges on the main scarp face. The Edale Shales are typical deep basinal deposits consisting of mudstones but occasionally siltstone and diagenetic carbonates occur. The carbonates take the form of either continuous beds or discrete concretions of dolomitic composition. Diagenetic pyrite occurs at several horizons, generally as scattered crystalline aggregates. Both shales and sandstones dip north-eastwards at approximately 5° on the main scarp face, but within slide units, dips approach 55° resulting from back tilting on the curved surfaces.

Mining of lead has been carried out on and around the Mam Tor area since Roman times and the oldest named mine is Odin Mine which was first recorded around 1280 A.D. (Ford & Rieuwerts 1976). As a result of this mining the stream known as Odin Sitch was diverted in the 18th century. The stream would normally have taken a course down the gorge following the line of the fault but the stream was redirected to flow north and then east around the mine and onto the landslide in order to prevent

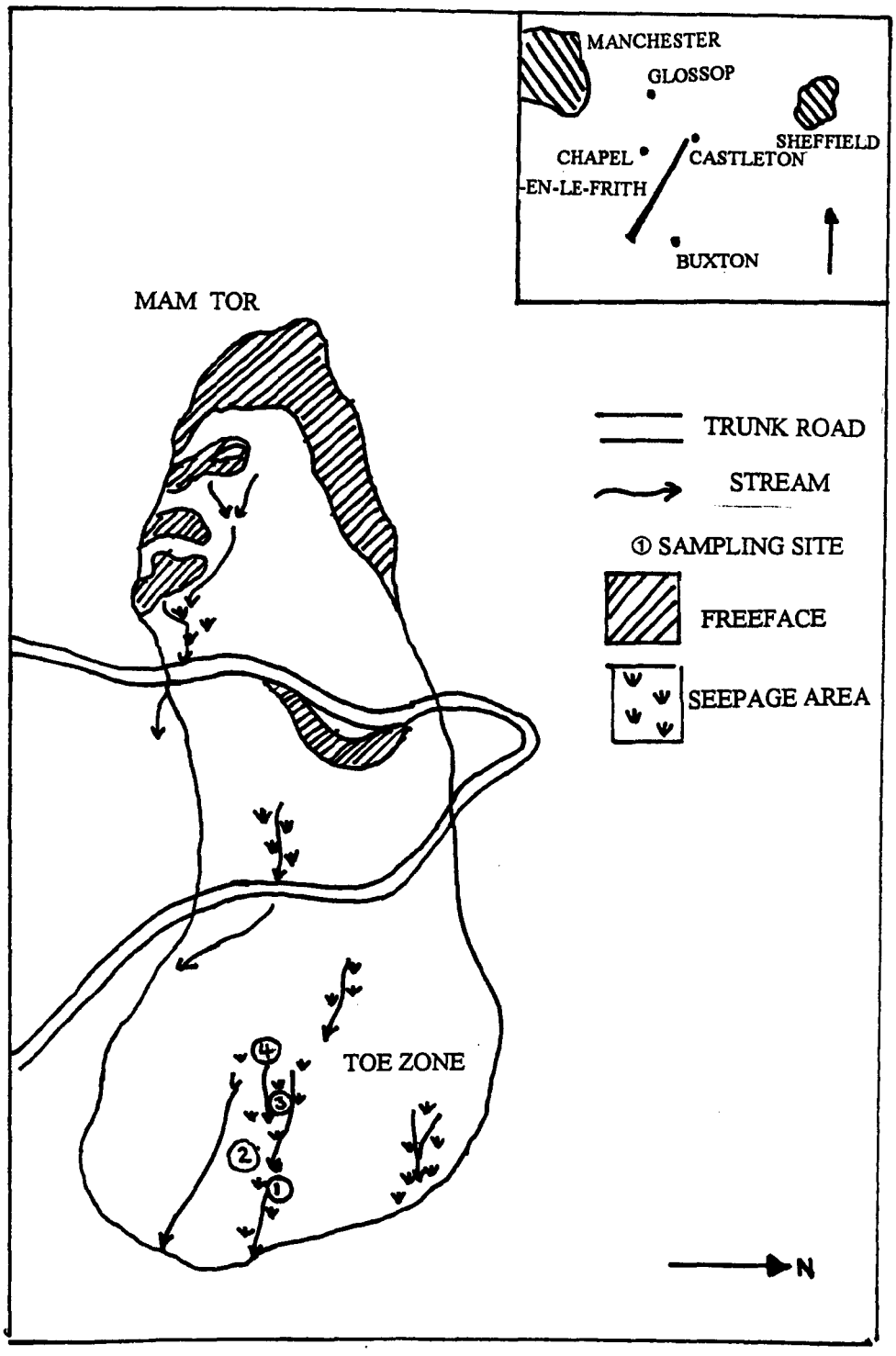


Figure 4.2. Location map of Mam Tor.

Plate 4.5. Main landslide at Mam Tor.

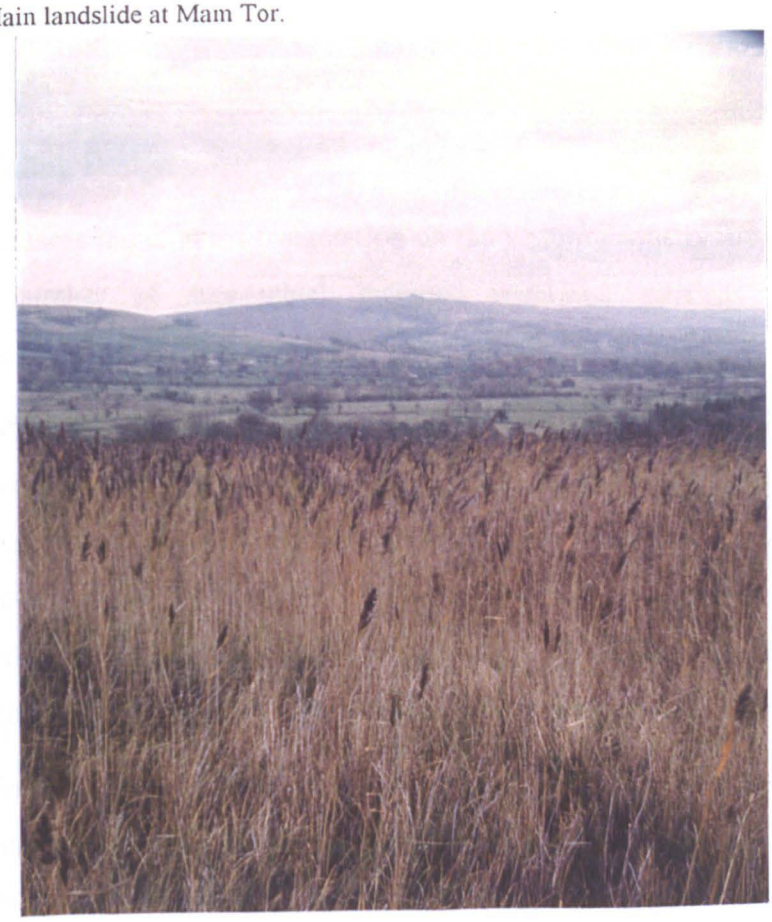


Plate 4.6. *P. australis* growth at Mam Tor.

flooding. However, the weathering of minerals exposed at Odin Rake does not contribute contaminants to the streams as any remaining mineralisation is below the Edale Shales (Vear 1981).

The toe region of the slide is characterised by high groundwater level, and the seasonal range of magnitude suggests that the winter recharge though partly derived from vertical infiltration is chiefly brought about by through flow of groundwater within the slide debris from higher parts of the landslide (Skempton *et al.* 1989). The high water table results in the hollows of the toe and these are charged with acid-sulphate 'ochre' waters (Vear & Curtis 1981). These waters originate from within the landslide where movement of material and subsequent aeration of the sulphide minerals has resulted in the oxidation of pyrite producing sulphuric acid which in turn liberates Fe, Mn, Cu and other elements present in the deposits. Waters flowing through the landslide are acidified, mobilise these elements and on exposure in the surface hollows precipitate iron oxide. In these iron-rich areas a large stand of *Phragmites australis* has become established together with other bog vegetation including *Sphagnum* species (Plate 4.6).

4.2.2. Sampling Design

In order to assess the effects of vegetation on the water chemistry it was necessary to choose a number of sites which included vegetated, partially vegetated and unvegetated areas of the field locations.

At Parys Mountain the unvegetated area (Site 1) was situated in the centre of the outlet stream adjacent to the vegetated areas. The presence of two vegetation types also necessitated the comparison of water chemistry between them, and so in each vegetated area a site at the centre and at the proximal edge was selected (Figure 4.1). Site 2 was the margin of the *P. australis* area, site 3 the central *P. australis* area, site 4 the margin of the *E. angustifolium* area and site 5 the central *E. angustifolium* area.

At Mam Tor (Figure 4.2), an unvegetated site was selected at the top of the slope. Samples were also collected at the base of the slope where a seepage area populated with *Juncus effusus* was situated (site 1), in the centre of the main *P. australis* colony

close to a seepage area (site 3) and at the lower edge of the *P. australis* colony (site 2).

Samples were collected every three months for the total period of one year, to allow for effects of seasonality. All samples were taken between the hours of 10.00 a.m and 1.00 p.m. in order to minimise the effects of chemical changes that occur throughout the day. The two sites were visited within 3 days of each other to allow direct comparison of seasonal measurements.

4.2.3. Surface Water Chemistry

At each site, 5 replicate water samples were collected by submerging clean, acid 1L plastic bottles with air tight caps in the streams or standing water. Each sample was filtered through two Whatman No.1 filters on site, one portion being acidified with 30μL of 30% HNO_3 to prevent the precipitation of iron and other metals. The unacidified sample was used to measure anion concentrations. All samples were kept at 4°C and were analysed within 7 days.

Five replicate field measurements of pH, oxidation-reduction potential and temperature were made at the time of sample collection at each site. pH was measured using a portable battery-operated meter (Jenway model 3100 microprocessor) which corrects for temperature effects. The instrument was calibrated with a buffer solution of pH 4.0 and 7.0.

Redox was measured using a portable battery-operated Pt-electrode multimeter (Hansen HD-31) which was calibrated in the field. In some cases however, it was found impossible to measure redox due to instability in the system. All redox readings were adjusted for pH in accordance to the guidelines set out in Golterman *et al.* 1978.

4.2.4 Porewater Chemistry

In order to investigate the distribution of cations and anions within the sediments, three replicate cores were taken at each site using a stainless steel cylindrical corer, 50 cm in length. Each core was sectioned on site into 5 cm lengths which were placed

clean plastic bags. All air was expelled from the bags which were stored at 4°C until porewater extraction (Chapter 2.3.1). Notes were taken on the physical appearance of the cores and the location of the rooting zone where applicable.

4.2.5. Statistical Analysis

Due to difficulties in the collection and analysis of porewaters from the cores obtained from the natural wetland systems it was difficult to obtain meaningful statistical analysis in all cases. However, where there was enough data a General Linear Model was used for two-way analysis of variance which allowed for unequal replication. This was followed by a multiple comparison Tukey test. Where data did not fit the assumptions of the GLM, the data was log transformed either by $\log_e$ or $\log_{10}$. When insufficient data were available for statistical analysis, general observations are made from the graphical plots of the profiles. Specifics of all statistical analyses are tabulated in appendix E. In all cases the coefficient of variance did not exceed 1.7%.

4.3 Results

4.3.1 Parys Mountain

4.3.1.1 Stream Water Chemistry

	Winter		Spring		Summer		Autumn	
	pH	Eh	pH	Eh	pH	Eh	pH	Eh
Site 1	3.0 ±.02	232.4 ±1.45	2.96 ±.01	238.8 ±1.3	2.93 ±.004	236.8 ±.23	2.59 ±.003	256.25 ±.17
Site 2	3.3 ±.02	369.4 ±6.6	3.06 ±.02	228.7 ±1.2	2.9 ±.002	238.1 ±.12	2.93 ±.006	236.24 ±.35
Site 3	3.51 ±.01	324.8 ±1.4	3.12 ±.01	225.0 ±.7	3.08 ±.003	227.8± .18	3.03 ±.006	230.92 ±.35
Site 4	2.93 ±.004	236.3 ±.26	2.89 ±.02	238.6 ±1.0	2.78 ±.007	245.0 ±.39	2.51 ±.005	261.13 ±.31
Site 5	2.99 ± .0004	232.6 ±.23	2.56 ±.007	258.1 ±.39	2.84 ±.01	241.6 ±.65	2.43 ±0	265.55 ±.005

Table 4.1. Chemistry of surface waters from Parys mountain. Means ± SE, n=5.

4.3.1.2 Porewater Chemistry

The rooting depth of both the *P. australis* and *E. angustifolium* occurred at a depth of 10 to 20 cm.

Iron

In winter there was a significant effect of site on Fe concentration in porewaters ($F=13.7$, $p<0.001$). Concentrations of Fe were higher in site 4 than other areas. Concentration of Fe also increased towards the surface of the profile although this was not a statistically significant result. In addition both sites 2 and 3 had higher concentrations than site 1.

In spring there was a significant effect of site ($F=20.9$, $p<0.001$) on Fe concentration in porewaters. Fe concentrations were higher in samples from the site 3 and 5 than 1 and 4. There was no significant effect of depth although concentrations increased down the profile from site 5 except for a sharp trough at 35 cm.

In summer there was a significant effect of site ($F=29.9$, $p<0.001$) on Fe concentration in porewaters with higher values recorded at site 3. Concentrations in sites 4 and 5 were also significantly higher than 1 and 2. There was a peak in Fe concentration at a depth of 20 cm in sites 4 and 5, and in site 3 concentrations increased rapidly just below the sediment surface at a depth of 10 cm.

In autumn there was a significant effect of site ($F=38.3$, $p<0.001$) and the interaction between site and depth ($F=1.7$, $p<0.05$) on Fe concentration in porewaters. Site 5 had higher concentrations than sites 2, 3 and 4. Concentrations of Fe increased in the base 10 cm of the profiles in sites 1 and 5 (Figure 4.3).

- Overall Fe concentrations were lower in autumn than the other seasons.
- Fe concentrations were consistently higher in the central *P. australis* zone than in the unvegetated site.
- Generally, concentrations increased down the profiles but there was significant variation within the cores.

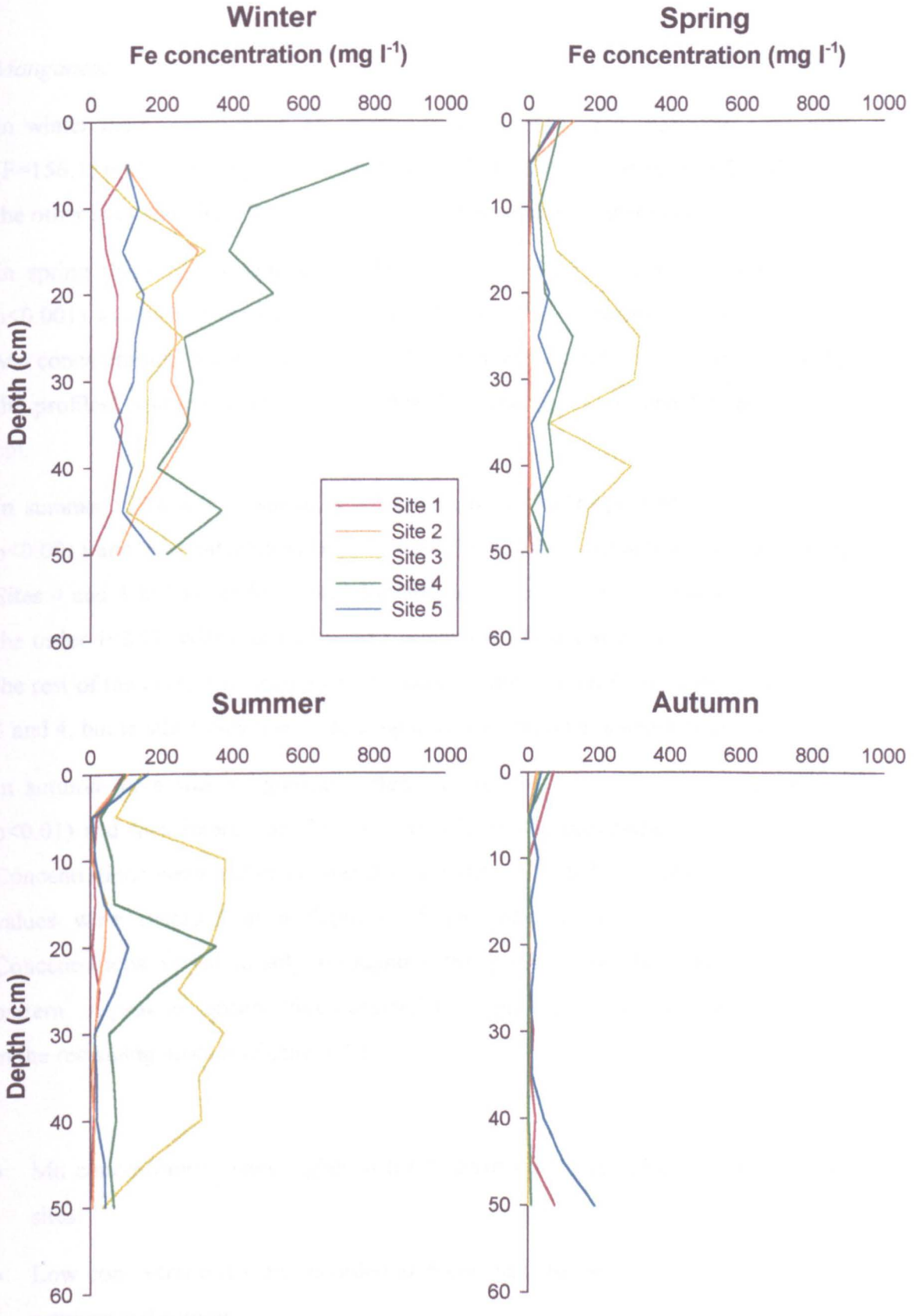


Figure 4.3. Fe concentration profiles at sampling sites, (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

Manganese

In winter there was a significant effect of site on Mn concentration in porewaters ($F=156.1$, $p<0.001$). Higher concentrations of Mn were found in sites 2 and 3 than the other sites and Site 5 had lower concentrations than the other sites.

In spring there was a significant effect of site ($F=92.8$, $p<0.001$), depth ($F=4.6$, $p<0.001$) and their interaction ($F=2.9$, $p<0.001$) on Mn concentration in porewaters. Mn concentrations were greater in samples from sites 2 and 3 than 1 and 5. Within the profiles, concentrations were significantly higher at 25, 30 and 50 cm than at 5 cm.

In summer there was a significant effect of site ($F=367.6$, $p<0.001$), depth ($F=5.6$, $p<0.001$) and their interaction ($F=1.7$, $p<0.05$) on Mn concentration in porewaters. Sites 4 and 5 had lower Mn concentrations than sites 1, 2 and 3, which increased in the order $1<2<3$. Within profiles lower values were found at a depth of 5 cm than in the rest of the core. Concentrations increase rapidly at a depth of 10 cm in both sites 3 and 4, but in site 3 only there was a dip in concentration at a depth of 20 cm.

In autumn there was a significant effect of site ($F=92.6$, $p<0.001$), depth ($F=2.7$, $p<0.01$) and their interaction ($F=2.6$, $p<0.001$) on Mn concentration in porewaters. Concentrations were higher in sites 2 and 3 than in 4 and 5. Within profiles lower values were recorded at a depth of 5 cm than at 20, 35, 45 and 50 cm. Concentrations varied greatly throughout the profiles but there was no distinct pattern. A peak in concentration occurred at 15 cm in site 1 but this was not evident in the remaining profiles (Figure 4.4).

- Mn concentrations were higher in the *P. australis* vegetated sites than the other sites.
- Low concentrations were recorded at 5 cm than the rest of the cores in spring, summer and autumn.
- Greatest variability in Mn concentration occurred in autumn.

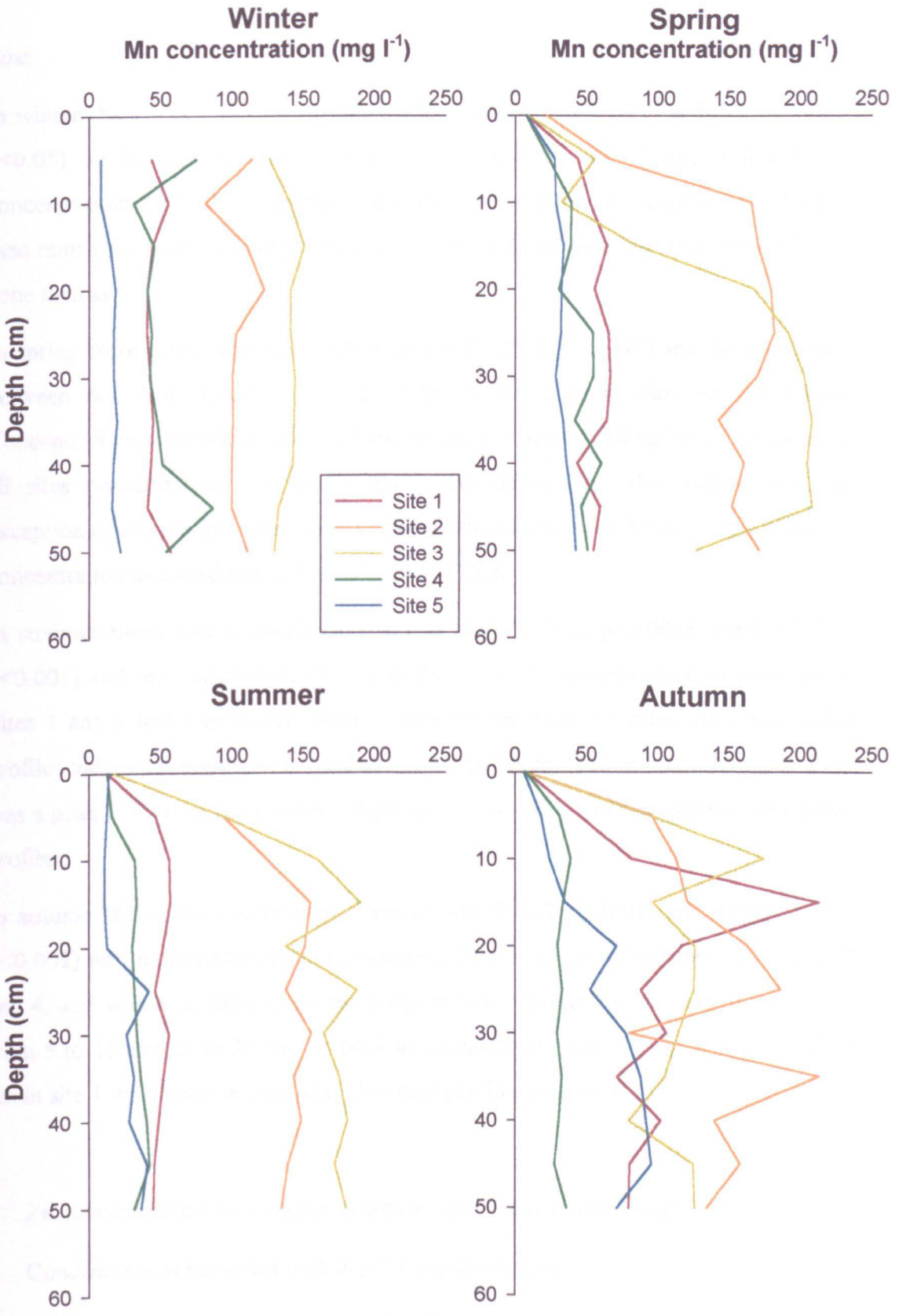


Figure 4.4. Mn concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

Zinc

In winter there was a significant effect of site ($F=43.5$, $p<0.001$) and depth ($F=2.6$, $p<0.05$) on Zn concentration in porewaters. Overall sites 2 and 3 had higher concentrations of Zn than the other sites. Zn concentrations were lower sites 4 and 5 than remaining sites. Within profiles, Zn concentrations were higher in the 40-50 cm zone than at 5 cm.

In spring there was a significant effect of site ($F=26.1$, $p<0.001$) and the interaction between site and depth ($F=1.9$, $p<0.05$) on Zn concentration in porewaters. Concentrations were higher in sites 2 and 3 than in 1 and 5, and higher in 1 than 5. In all sites concentration increased slightly with depth from the surface with the exception of site 4 where there was a sharp decline in the base 5 cm. A small peak in concentration occurred in site 5 at a depth of 15 cm.

In summer there was a significant effect of site ($F=70.2$, $p<0.001$), depth ($F=5.2$, $p<0.001$) and their interaction ($F=2.3$, $p=0.001$) on Zn concentration in porewaters. Sites 1 and 5 had significantly lower concentrations than the other sites and within profiles values were greater at depths from 35 to 50 cm than at 5 cm. In site 5 there was a peak in Zn concentration at a depth of 20 cm which was not evident in the other profiles.

In autumn there was a significant effect of site ($F=4.2$, $p<0.01$) and depth ($F=5.7$, $p<0.001$) on Zn concentration in porewaters. Site 3 had lower concentrations than 2 and 4, and within profiles values were higher at a depth of 50 cm than in the ranges from 5 to 15 and 25 to 30 cm. A peak in concentration was evident at a depth of 45 cm in site 1 which was not found in the other profiles (Figure 4.5).

- Zn concentrations were higher in autumn that the rest of the year.
- Concentrations increased with depth from the surface.
- Higher concentrations were recorded in the central *P. australis* site than the others.

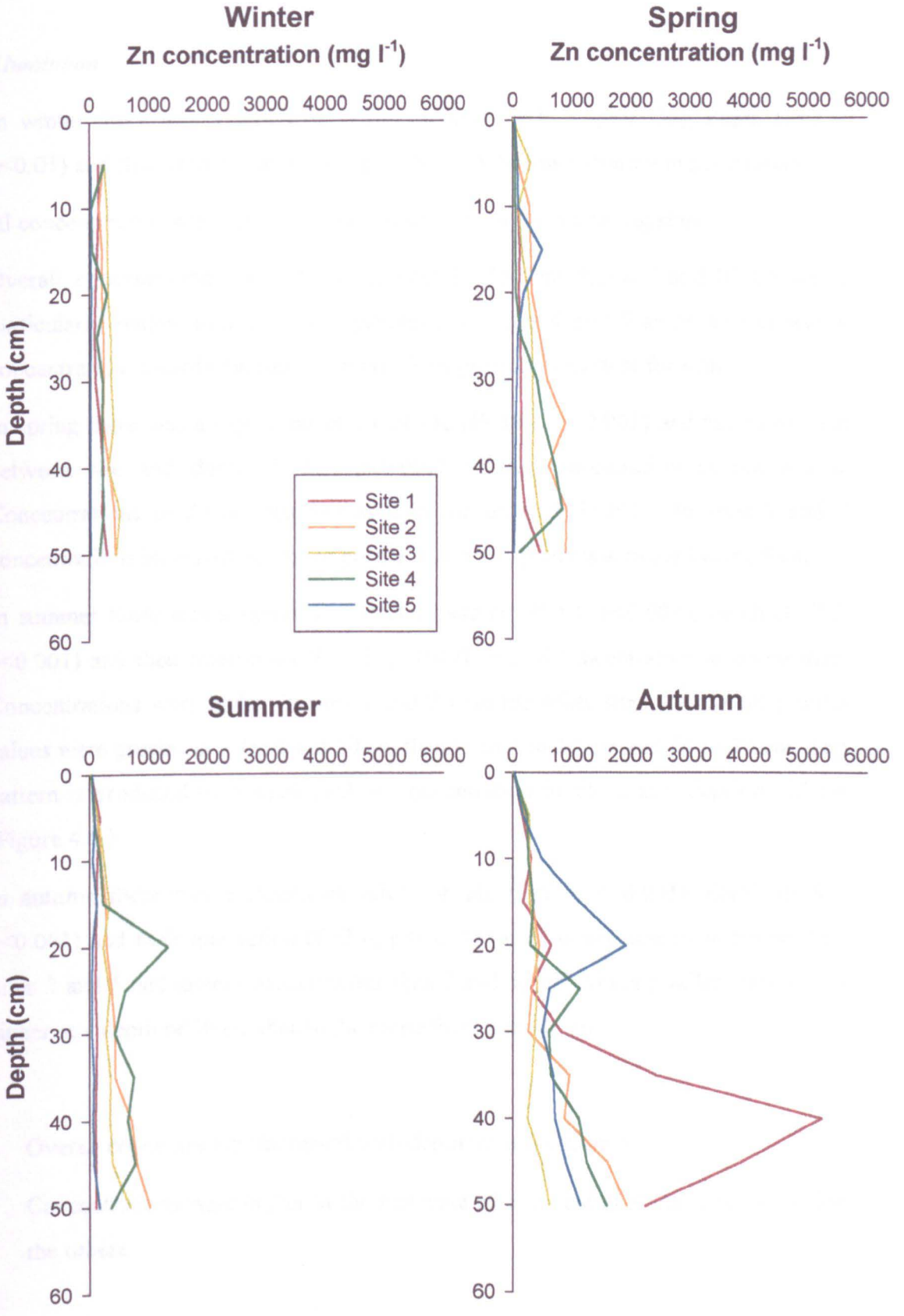


Figure 4.5. Zn concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

Aluminium

In winter there was a significant effect of site ($F=88.5$, $p<0.001$), depth ($F=2.9$, $p<0.01$) and their interaction ($F=3.9$, $p<0.001$) on Al concentration in porewaters.

Al concentrations site 1, 2 and 3 were higher than in the remaining sites.

Overall, concentrations were greater at a depth of 50 cm than at 5 and 10 cm, this is particularly evident in site 1. The profiles from sites 4 and 5 show an increase in concentration towards the top of the profile as previously evident for iron.

In spring there was a significant effect of site ($F=48.1$, $p<0.001$) and the interaction between site and depth ($F=2.7$, $p<0.001$) on Al concentration in porewaters. Concentrations of Al in sites increased in the order $5<3<2<1$. In sites 1 and 2 concentrations increased down the profile with a sharp increase in the lowest 5 cm.

In summer there was a significant effect of site ($F=200.4$, $p<0.001$), depth ($F=7.2$, $p<0.001$) and their interaction ($F=8.5$, $p<0.001$) on Al concentration in porewaters. Concentrations were higher in sites 1 and 2 than the other sites and within profiles values were greater at a depths of 50 cm than from 5 to 10 cm and 20 to 30 cm. This pattern is produced by a small peak in concentration in site 2 at a depth of 15 cm (Figure 4.6).

In autumn there was a significant effect of site ($F=6.3$, $p<0.001$), depth ($F=8.2$, $p<0.001$) and their interaction ($F=3.6$, $p<0.001$) on Al concentration in porewaters. Sites 3 and 5 had lower concentrations than 2 and 4, and within profiles values were higher at a depth of 50 cm than in the range from 5 to 40 cm.

- Overall concentrations increased with depth from the surface.
- Concentrations were higher in the unvegetated and central *P. australis* sites than the others.

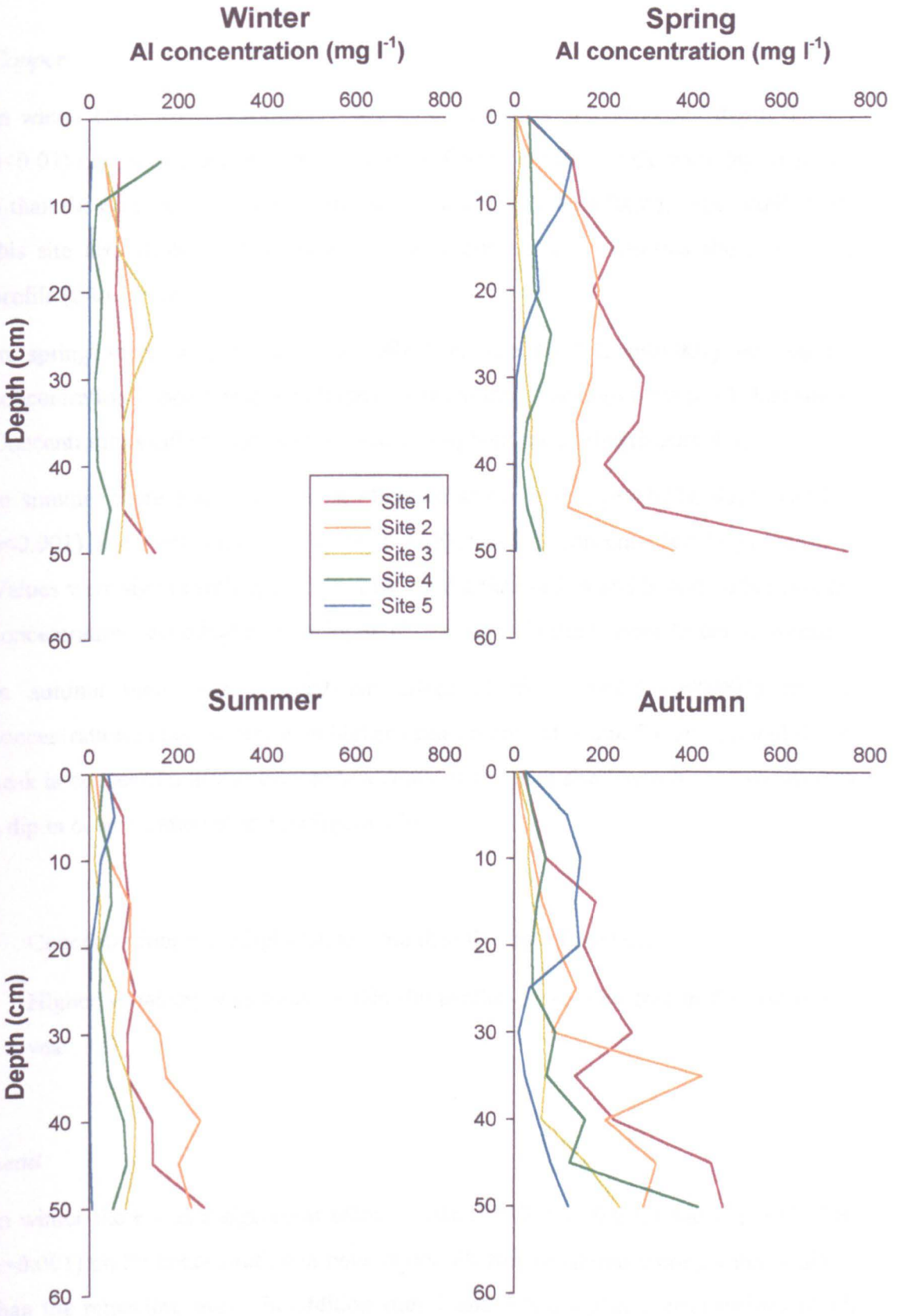


Figure 4.6. Al concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

Copper

In winter there was a significant effect of site ($F=27.8$, $p<0.001$) and depth ($F=3.3$, $p<0.01$) on Cu concentration in porewaters. Concentrations of Cu were higher in site 4 than the other sites. No other significant differences were found. The profile from this site also showed an increase in copper concentration towards the top of the profile (0 to 10 cm).

In spring there was a significant effect of site ($F=3.7$, $p<0.001$) on the Cu concentration in porewater with higher values found in samples from site 1 than site 3. Concentrations did not vary significantly throughout the profile (Figure 4.7).

In summer there was a significant effect of site ($F=16.1$, $p<0.001$), depth ($F=4.2$, $p<0.001$) and their interaction ($F=3$, $p<0.001$) on Pb concentration in porewaters. Values were significantly greater in sites 1 and 2 than in 3, 4 and 5, and within profiles concentrations were highest at a depth of 15 cm than in the bottom 15 cm of the core

In autumn there was a significant effect of site ($F=62.5$, $p<0.001$) on Cu concentration in porewaters with higher values recorded in site 5 than 2, 3 and 4. A peak in concentration was evident at a depth of 30 cm in site 1 which corresponded to a dip in concentration at site 5 (Figure 4.7).

- Concentrations were higher in autumn than the rest of the year.
- Higher variability was found within the profiles in autumn that in the rest of the year.

Lead

In winter there was a significant effect of site ($F=19.8$, $p<0.001$) and depth ($F=3.8$, $p=0.001$) on Pb concentration in porewaters Pb concentrations were greater in site 4 than the remaining sites. In addition sites 1 and 3 had higher concentrations of Pb than site 5. Within the profiles, Pb concentrations were lower at 40 and 50 cm than in the zone from 5 to 15 cm. From the graph (Figure 4.8) it was evident that in

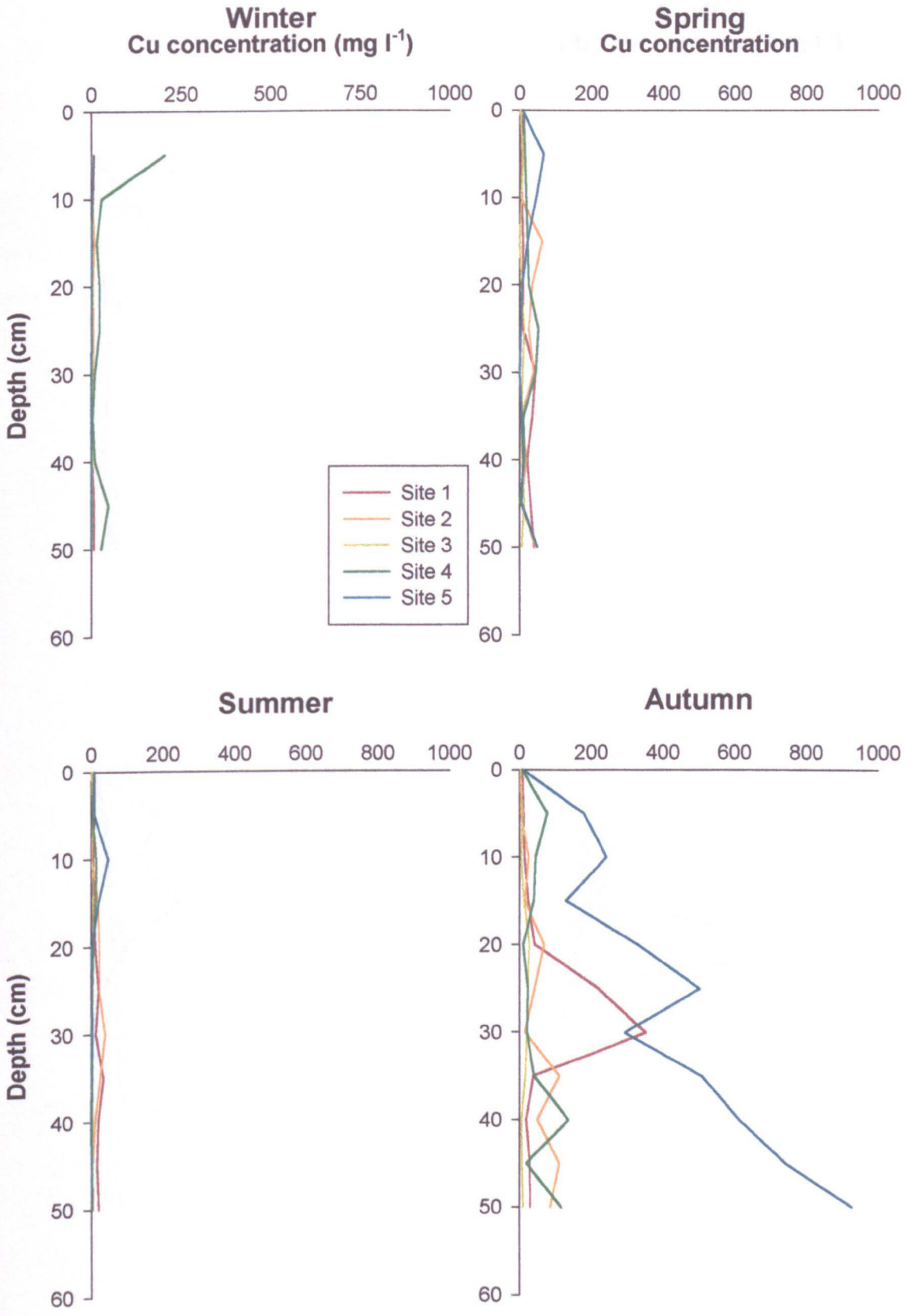


Figure 4.7. Cu concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

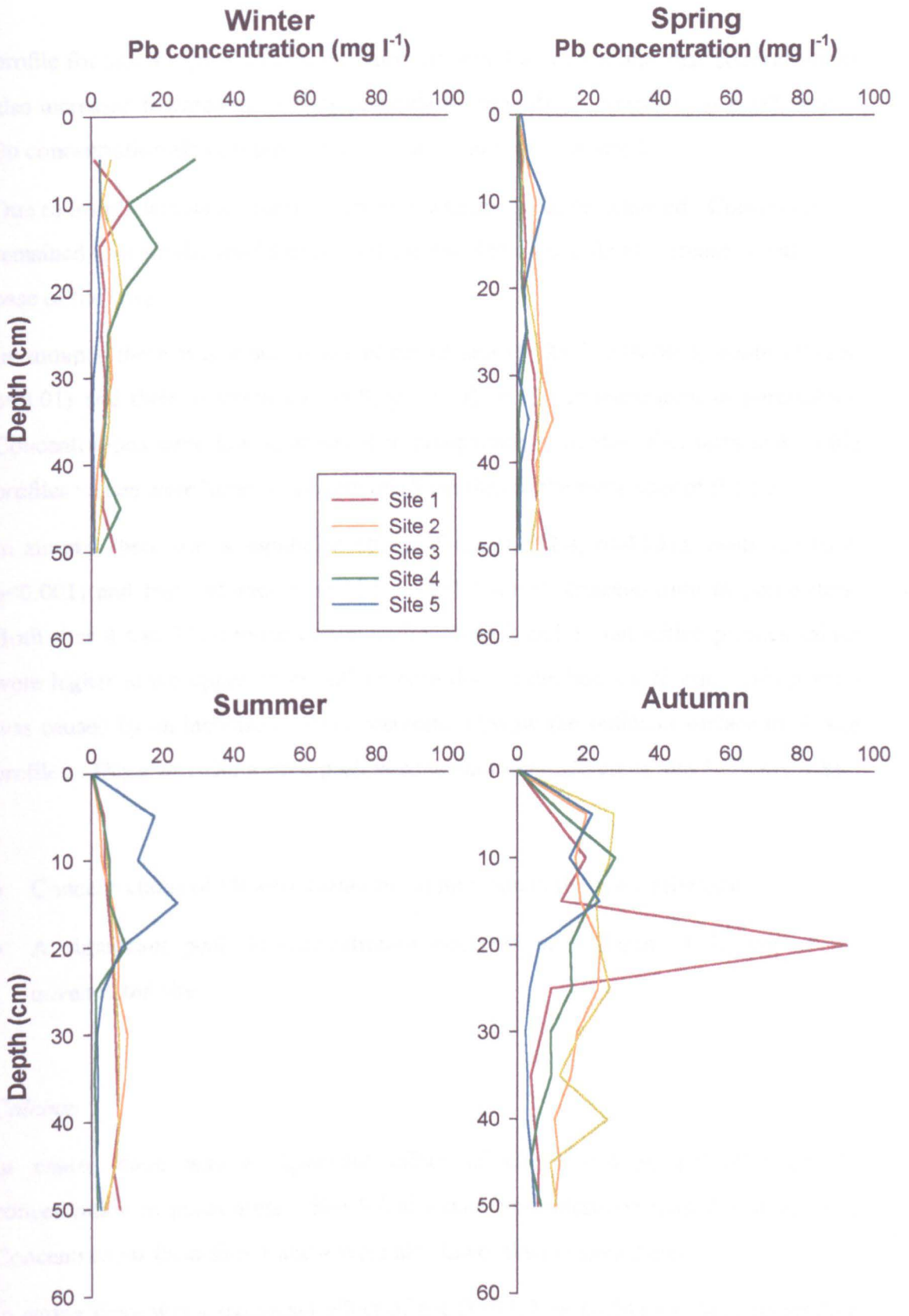


Figure 4.8. Pb concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

profile for site 4 a peak of concentration occurred at 15 cm and that concentrations also increased towards the surface particularly from 10 cm upwards. A small peak in Pb concentration also occurred at 10 cm in the profile from site 1.

Due to insufficient data, statistical analysis was unable to be achieved. Concentrations remained at a similar level throughout the profiles with a slight increase towards the base of the core.

In summer there was a significant effect of site ($F=21.7$, $p<0.001$), depth ($F=2.8$, $p<0.01$) and their interaction ($F=3.8$, $p<0.001$) on Cu concentration in porewaters. Concentrations were lowest at site 4 in comparison with the other sites and within profiles values were lower at a depth of 15 cm than in the remainder of the core.

In autumn there was a significant effect of site ($F=32.4$, $p<0.001$), depth ($F=10.7$, $p<0.001$) and their interaction ($F=1.8$, $p<0.05$) on Pb concentration in porewaters. Both sites 4 and 5 had lower concentrations than 2 and 3, and within profiles values were higher in the upper 15 cm of the core than in the bottom 20 cm. This pattern was caused by an increase in Pb concentration below the sediment surface in all site profiles. There was also a large peak in concentration at 20 cm in site 1 (Figure 4.8).

- Concentrations of Pb were higher in autumn than in the rest of the year.
- A significant peak in concentration occurred at a depth of 20 cm in the unvegetated site.

Calcium

In winter there was a significant effect of site ($F=145.9$, $p<0.001$) on Ca concentration in porewaters. Site 5 had lower concentrations than the other sites. Concentrations from sites 1 and 4 were also lower than in sites 2 and 3.

In spring there was a significant effect of site ($F=11.9$, $p<0.001$) on Ca concentration in porewaters with lower values found in samples from site 5 than the other sites. Concentrations varied greatly down the profiles of all sites but there was no general

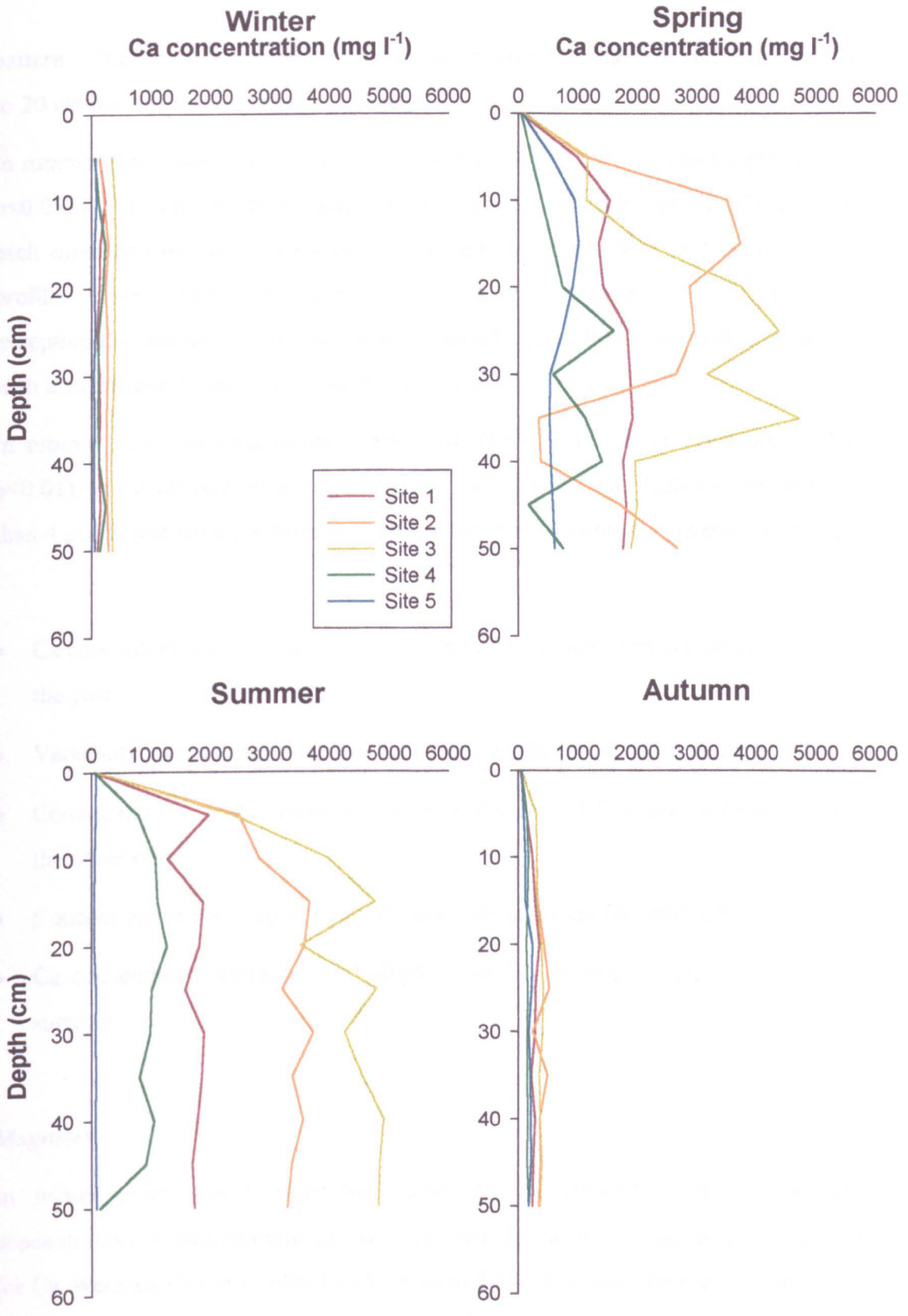


Figure 4.9. Ca concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

pattern. Concentrations appeared to increase in sites 1, 2 and 5 in the zone from 10 to 20 cm but this was not clearly defined.

In summer there was a significant effect of site ($F=286.7$, $p<0.001$) and depth ($F=2.3$, $p<0.05$) on Ca concentration in porewaters. All sites were significantly different from each other and increased in Ca concentration in the order $5<4<1<2<3$, and within profiles values were higher at depths of 15 and 40 cm than at 5 cm. In all profiles excepting that for site 5, concentrations increased rapidly below the sediment surface with a significant dip in concentration at 25 cm in site 3 (Figure 4.9).

In autumn there was a significant effect of site ($F=76.7$, $p<0.001$) and depth ($F=2.7$, $p<0.01$) on Ca concentration in porewaters. Site 2 and 3 had higher concentrations than 4 and 5, and within profiles values were lower at a depth of 5 cm than at 25 cm.

- Ca concentrations were higher in the spring and summer months than in the rest of the year
- Variability within the profiles was also higher in the spring and summer months.
- Concentrations were consistently lower in the central *E. angustifolium* site than the other sites.
- Concentrations were higher in the *P. australis* sites than the other sites.
- Ca concentration increased with depth from the sediment surface in spring and summer.

Magnesium

In winter there was a significant effect of site ($F=89.7$, $p<0.001$) on Mg concentration. Concentrations of Mg were lower in the site 5 than the others and as for Ca, were also lower in sites 1 and 4 than in 2 and 3. Within the profile from site 4 there is an increase in Mg concentration towards the top (0 to 10 cm) but this was not statistically significant.

In spring there was a significant effect of site ($F=165$, $p<0.001$), depth ($F=11.4$, $p<0.001$) and their interaction ($F=7.5$, $p<0.001$) on Mg concentration in porewaters.

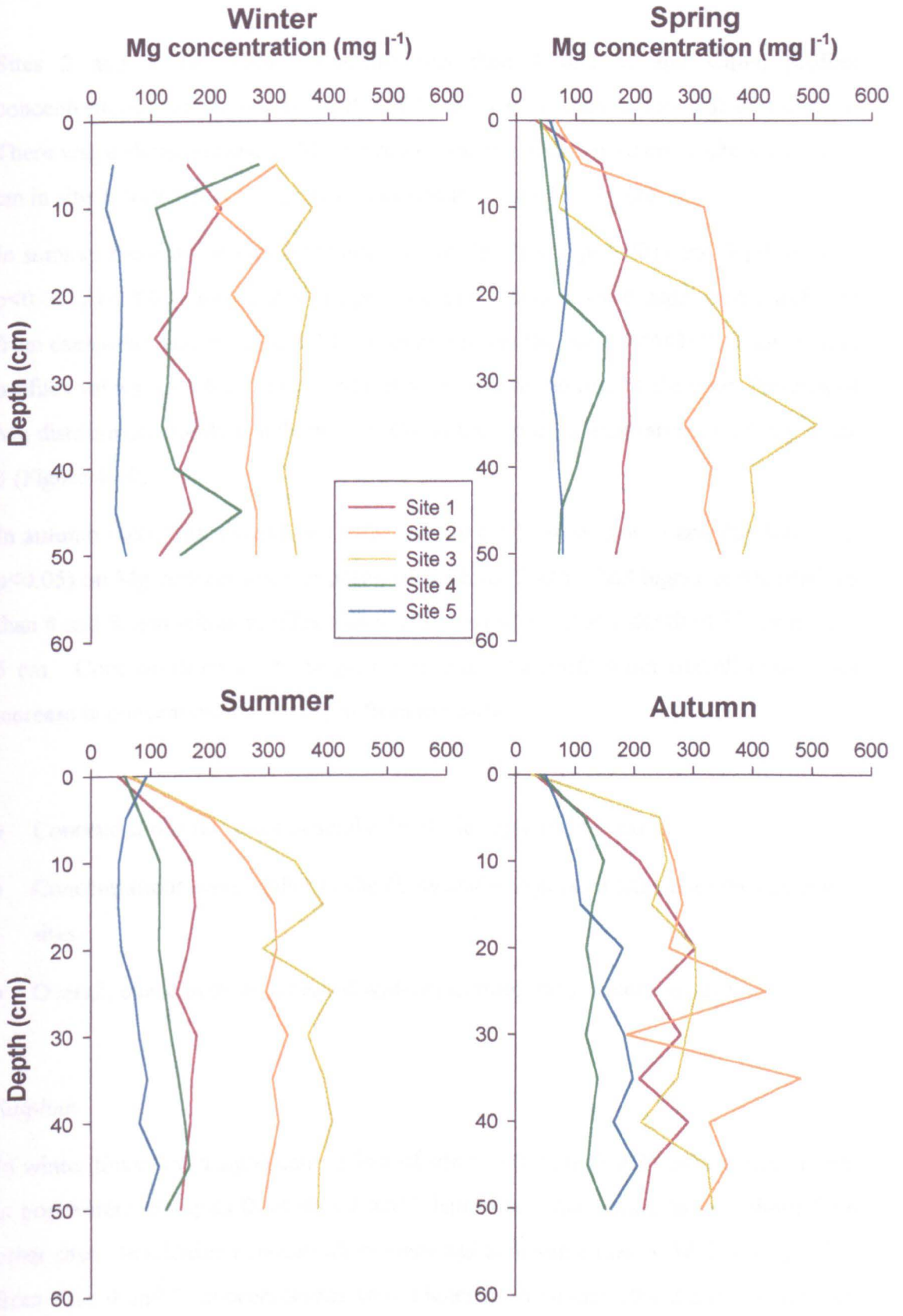


Figure 4.10. Mg concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

Sites 2 and 3 had higher concentrations than 1 and 5, and within profiles concentrations were lower at depths of 5 and 10 cm than in the rest of the core. There was a sharp increase in Mg concentration at a depth of 10 cm in site 2 and at 20 cm in site 3, together with a peak in concentration at 35 cm in site 3.

In summer there was a significant effect of site ($F=268.9$, $p<0.001$) and depth ($F=6.8$, $p<0.001$) on Mg concentration in porewaters. All sites were significantly different from each other and increase in Mg concentration in the order $5<4<1<2<3$, and within profiles values were lower at a depth of 5 cm than in the rest of the core. Patterns of Mg distribution closely match those of Ca with a dip in concentration at 25 cm in site 3 (Figure 4.10).

In autumn there was a significant effect of site ($F=53.4$, $p<0.001$) and depth ($F=2.2$, $p<0.05$) on Mg concentration in porewaters. Sites 2 and 3 had higher concentrations than 4 and 5, and within profiles higher values were found at a depth of 35 cm than at 5 cm. Concentrations fluctuate greatly through the profiles but overall there is an increase in concentration with depth from the surface.

- Concentrations remained at similar levels throughout the year.
- Concentrations were higher in the *P. australis* vegetated sites than the remaining sites.
- Overall, concentration increased with depth from the sediment surface.

Sulphur

In winter there was a significant effect of site ($F=45.1$, $p<0.001$) on S concentration in porewaters Samples from sites 1 and 5 had lower concentrations than those from other sites. In addition concentrations were higher in site 4 than 5. Within the profiles from sites 4 and 5, concentrations were higher in the upper 10 cm but this was not statistically significant.

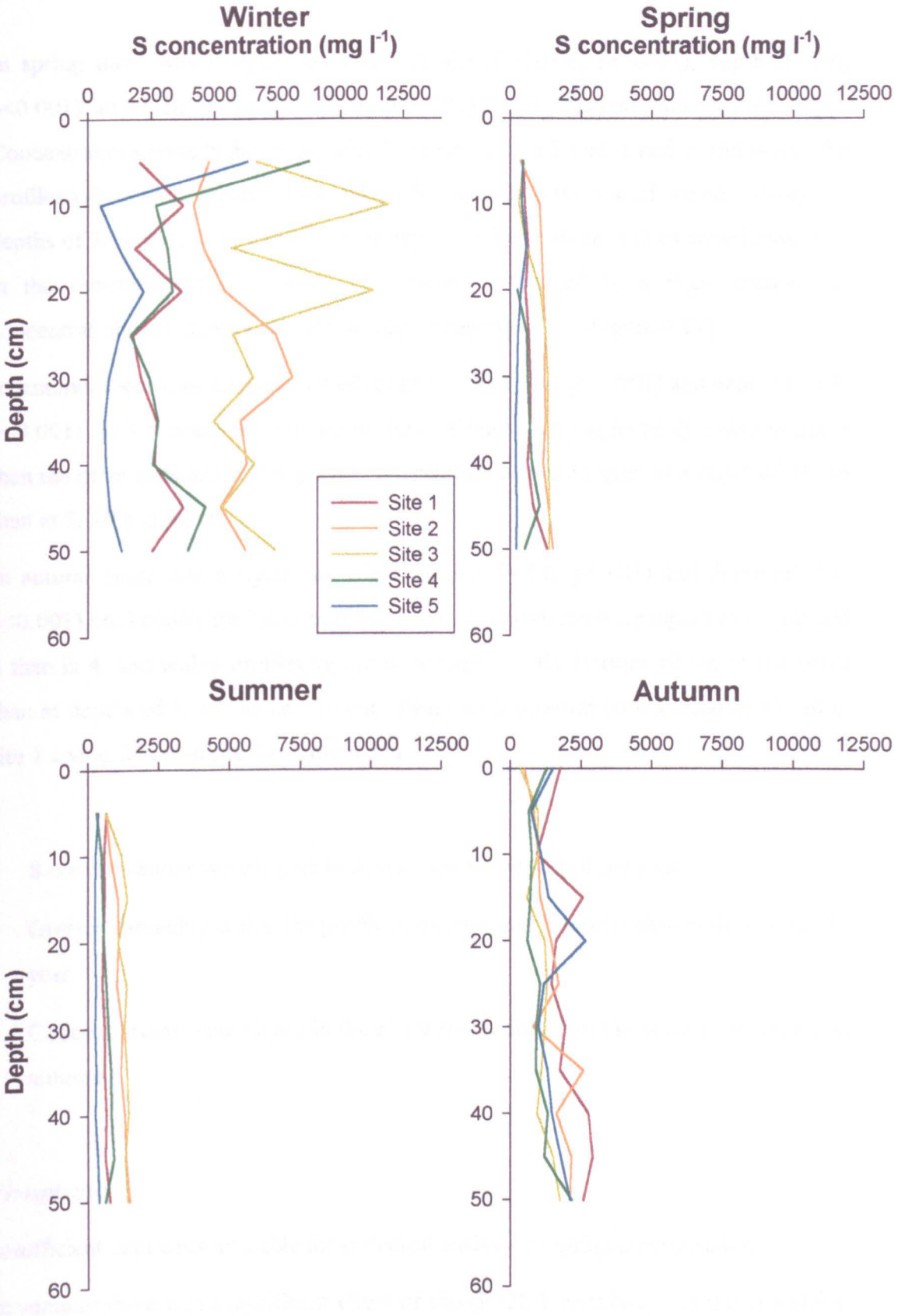


Figure 4.11. S concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

In spring there was a significant effect of site ($F=103.6$, $p<0.001$), depth ($F=9.6$, $p<0.001$) and their interaction ($F=5$, $p<0.001$) on S concentration in porewaters. Concentrations were higher in samples from sites 2 and 3 than 1 and 5, and within the profiles values were greater at a depth of 50 cm than in the rest of the core excepting depths of 30 and 45 cm. In addition at depths of 5 and 10 cm values were lower than in the remainder of the core. This pattern is caused by a slight increase in concentration with depth in all site profiles excepting site 5 (Figure 4.11).

In summer there was a significant effect of site ($F=135.5$, $p<0.001$) and depth ($F=4.9$, $p<0.001$) on S concentration in porewaters. Values were significantly lower in site 5 than the other sites and within profiles concentrations were higher at a depth of 45 cm than at 5, 10 and 20 cm.

In autumn there was a significant effect of site ($F=4.6$, $p<0.01$) and depth ($F=4.7$, $p<0.001$) on S concentration in porewaters. Concentrations were higher in sites 2 and 5 than in 4, and within profiles values were higher in the bottom 10 cm of the cores than at depths of 5, 10, 15 and 30 cm. Peaks in concentration occurred at 15 cm in site 1 and at 20 cm in site 5 (Figure 4.11).

- S concentrations were higher in winter than in the rest of the year.
- Greater variability within the profiles was recorded in winter than in the rest of the year.
- Concentrations were higher in the *P. australis* sites than the other sites, except in autumn.

Phosphorus

Insufficient data were available for statistical analysis of spring concentrations.

In summer there was a significant effect of site ($F=22.1$, $p<0.001$) and the interaction between site and depth ($F=3.4$, $p<0.001$) on P concentration in porewaters. Concentrations were lower in site 4 than in 2 and 3. There was a peak in

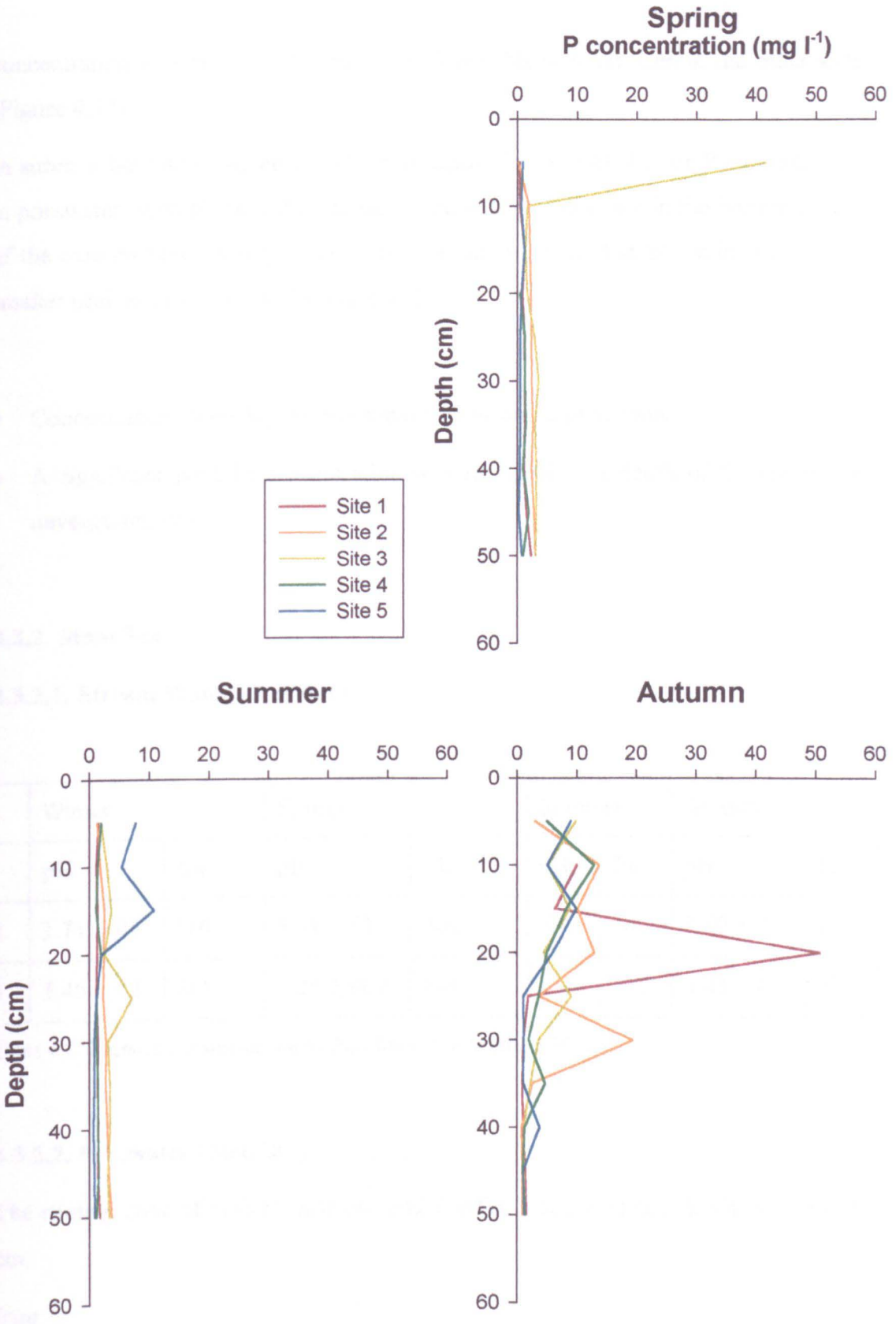


Figure 4.12. P concentration profiles at sampling sites (1) stream, (2) *P. australis* edge, (3) *P. australis* centre, (4) *E. angustifolium* edge and (5) *E. angustifolium* centre from Parys Mountain.

concentration at a depth of 15 cm in site 5 but this was not seen in the other sites (Figure 4.12).

In autumn there was a significant effect of depth ($F=9.4$, $p<0.001$) on P concentration in porewaters with higher values found in the upper 15 cm than in the bottom 20 cm of the core profiles. A large peak in concentration occurred at 20 cm in site 1 and a smaller peak at 30 cm in site 2 (Figure 4.12).

- Concentrations were higher in autumn than in spring or summer.
- A significant peak in concentration was recorded at a depth of 20 cm in the unvegetated site.

4.3.2. Mam Tor

4.3.2.1. Stream Water Chemistry

	Winter		Spring		Summer		Autumn	
	pH	Eh	pH	Eh	pH	Eh	pH	Eh
1	3.71 ±.03	NA	3.61 ±.01	NA	NA	NA	3.70 ±.03	NA
3	3.46 ±.02	NA	3.29 ±.002	NA	NA	NA	3.43 ±.02	NA

Table 4.2. Chemistry of surface waters from Mam Tor. Means ± SE, n=5.

4.3.2.2. Porewater Chemistry

The rooting zone of both *P. australis* and *J. effusus* occurred at a depth of 10 to 20 cm.

Iron

In spring there was a significant effect of site ($F=25.1$, $p<0.001$), depth ($F=13.5$, $p<0.001$) and their interaction ($F=2.7$, $p<0.001$) with concentrations in each site differing significantly from each other in the order $3<1<4$. Within the profiles

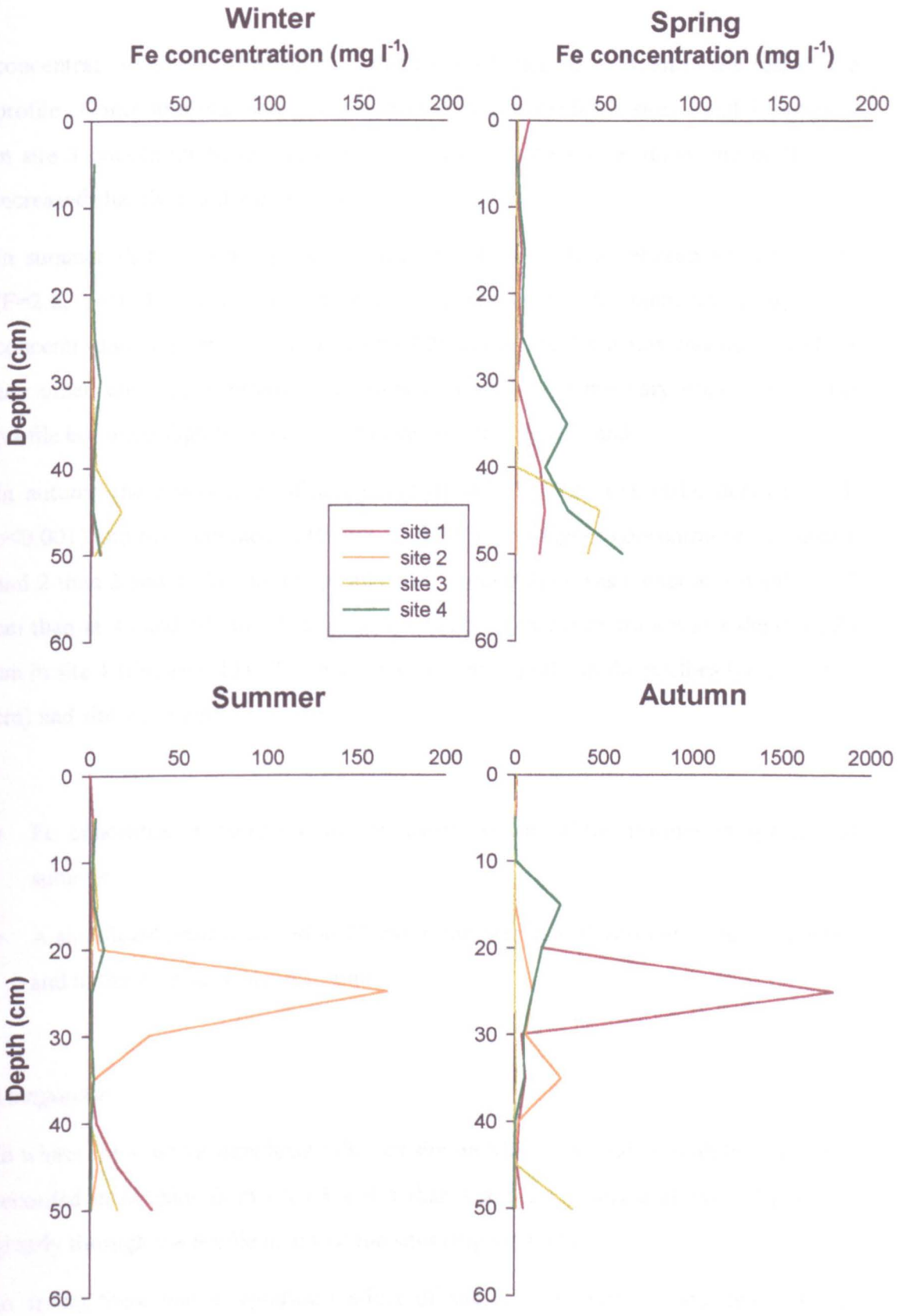


Figure 4.13. Fe concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

concentrations of Fe were higher at depths of 45 and 50 cm than in the rest of the profile. Concentrations increased gradually down the profile in sites 1 and 3, however in site 3 concentration remained at a relatively constant level down the profile but increased sharply at a depth of 45 cm.

In summer there was a significant effect of the interaction between site and depth ($F=2.2$, $p<0.01$) on Fe concentration in porewaters. A significant peak in Fe concentration was recorded at a depth of 25 cm in site 2 but this was not found for any other site. Concentrations for sites 1, 3 and 4 did not vary greatly down the profile but were slightly higher in the lower 10 cm in sites 1 and 3.

In autumn there was a significant effect of site ($F=12.6$, $p<0.001$), depth ($F=5.4$, $p<0.001$) and their interaction ($F=4.7$, $p<0.001$) with higher concentrations in sites 1 and 2 than 3 and 4. Within the profiles Fe concentration was higher at a depth of 25 cm than at 45 and 50 cm. There was a large peak in concentration at a depth of 25 cm in site 1 (Figure 4.13). There were also smaller peaks in the profiles for site 2 (35 cm) and site 4 (15 cm).

- Fe concentration increased in the lower 10 cm of the profiles in spring and summer.
- A significant peak occurred at 25 cm in the proximal *P. australis* site in summer, and in the *J. effusus* site in autumn.

Manganese

In winter there was a significant effect of site on Mn concentration with higher values recorded in samples from sites 3 and 4 than 1 and 2. Concentrations did not vary greatly through the profile in any of the sites (Figure 4.14).

In spring there was a significant effect of site ($F=3.7$, $p<0.05$) and depth ($F=11$, $p<0.001$) with higher concentrations of Mn found in samples from site 1 than site 3. Within the profiles higher concentrations were recorded at a depth of 50 cm than in the rest of the profile but this was not significantly greater than 45 cm.

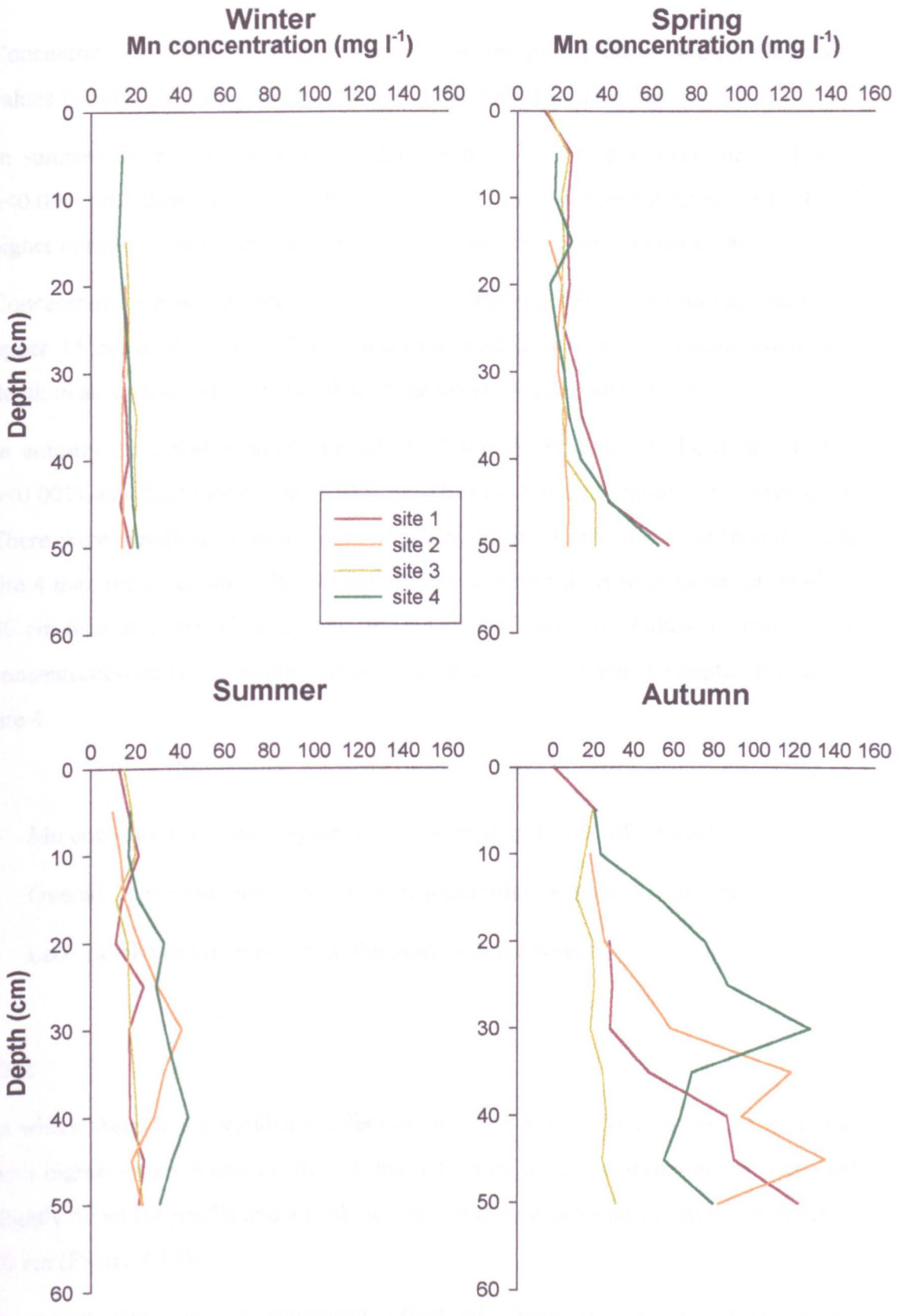


Figure 4.14. Mn concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

Concentrations of Mn increase gradually down the profile for all sites, with greater values found in the lower 10 cm in sites 1 and 4 than the other sites.

In summer there was a significant effect of site ($F=23.4$, $p<0.001$), depth ($F=6.6$, $p<0.001$) and their interaction ($F=2.9$, $p<0.001$) on Mn concentration. Site 4 had higher concentrations than site 2, and these were both higher than sites 1 and 3.

Concentrations within profiles were highest at depths of 30, 35 and 40 cm than in the upper 15 cm of the core. This reflects the gradual increase in concentration with depth in all sites and the small decline in the lower 10 cm (Figure 4.14).

In autumn there was a significant effect of site ($F=32.4$, $p<0.001$), depth ($F=5.2$, $p<0.001$) and their interaction ($F=2.6$, $p<0.01$) on Mn concentration in porewaters. There were significantly higher concentrations in site 3 and lower concentrations in site 4 than the other sites. Within the profiles concentrations were higher at depth of 50 cm than at either 20 or 25 cm. Figure 4.14 shows this gradual increase in Mn concentration down the profile and also a peak in concentration at a depth of 30 cm in site 4.

- Mn concentrations were higher in autumn than in the rest of the year.
- Overall, concentrations increased with depth from the sediment surface.
- Low variability occurred within the profiles in the winter.

Zinc

In winter there was a significant effect of site ($F=7.8$, $p<0.001$) on Zn concentration with higher values found in site 1,2 and 4 than in site 2. Concentrations increased slightly down the profile and a peak in concentration was found in site 4 at a depth of 40 cm (Figure 4.15).

In spring there was a significant effect of depth ($F=5.5$, $p<0.001$) on Zn concentration. Within the profiles values were greater at depths of 30, 45 and 50 cm

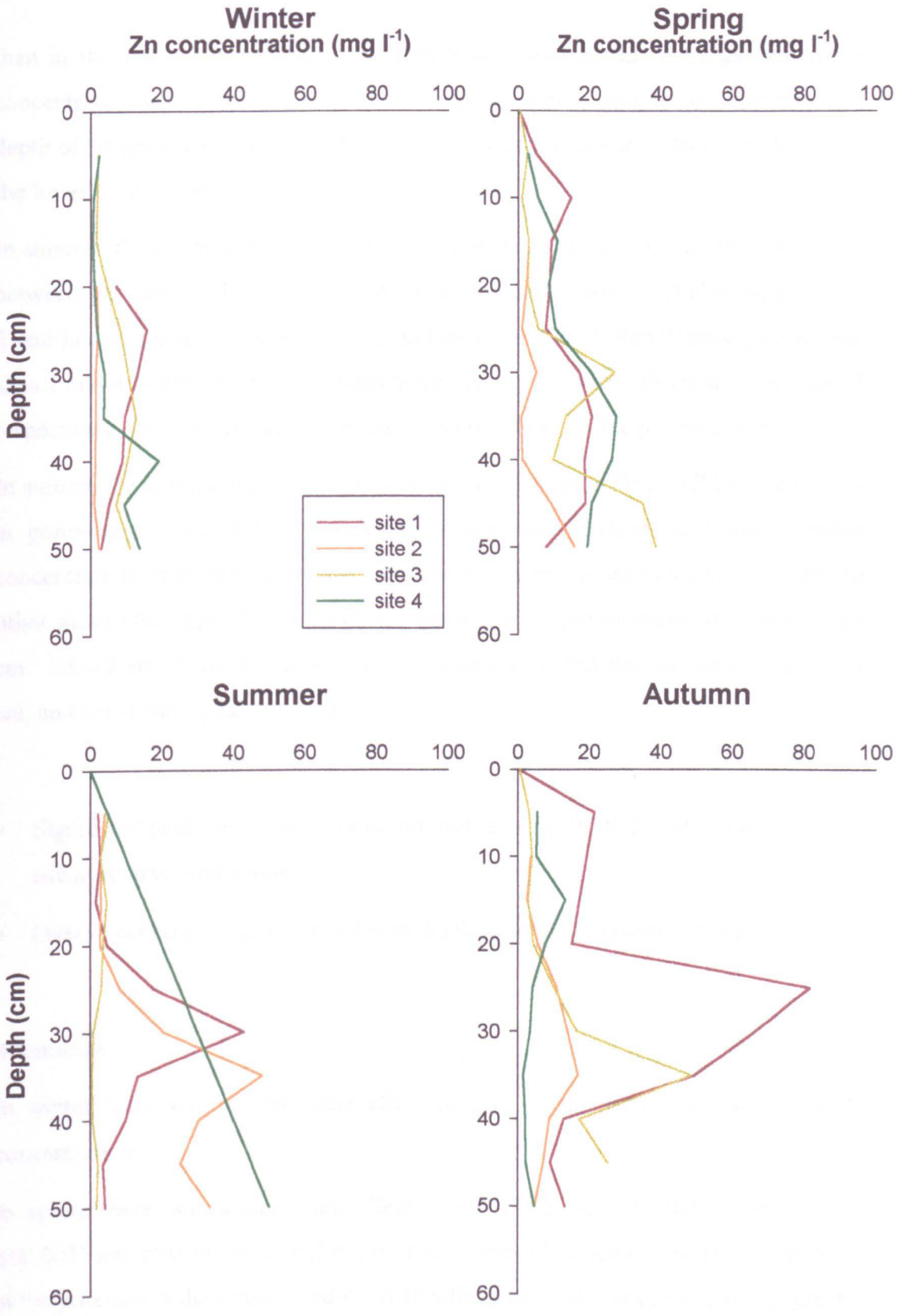


Figure 4.15. Zn concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

than in the zone from 5 to 25 cm. This pattern was produced by an increase in concentration with depth in sites 2 and 3, together with a peak in concentration at a depth of 30 cm again in sites 2 and 3. There was also a decline in Zn concentration in the lower 10 cm in sites 1 and 4.

In summer there was a significant effect of site ($F=33$, $p<0.001$) and the interaction between site and depth ($F=3.8$, $p<0.001$) on Zn concentration. Higher values were found in samples from site 3 than in 2 and these were both significantly greater than sites 1 and 4. Peaks in concentrations were evident at 30 and 35 cm in sites 1 and 3 respectively. No change in concentration occurred through the profile in site 3.

In autumn there was a significant effect of site ($F=15.5$, $p<0.001$) on Zn concentration in porewaters. Site 4 had significantly lower concentrations and site 1 higher concentrations than the other sites. In site 1 concentrations were higher than the other sites in the upper 20 cm, and there was a peak in concentration at a depth of 25 cm. Sites 2 and 3 also had a peak in Zn concentration but this was at a depth at 35 cm, and site 4 had a peak at 15 cm.

- Significant peaks occurred in concentration at a depth of 30 cm in the *J. effusus* site in summer and autumn.
- Overall concentrations increased with depth from the sediment surface.

Aluminium

In winter there was no significant effect of site, depth or their interaction on Al concentration.

In spring there was a significant effect of site ($F=12.7$, $p<0.001$), depth ($F=5.8$, $p<0.001$) and their interaction ($F=2$, $p<0.05$). Site 3 had lower concentrations of Al in the porewaters than sites 1 and 4. Within the profiles Al concentration was greater at a depth of 45 cm than in the zone from 5 to 25 cm. In addition values were lower at a depth of 5 cm than in the lower 15 cm. Concentrations declined gradually with increasing distance from the sediment surface.

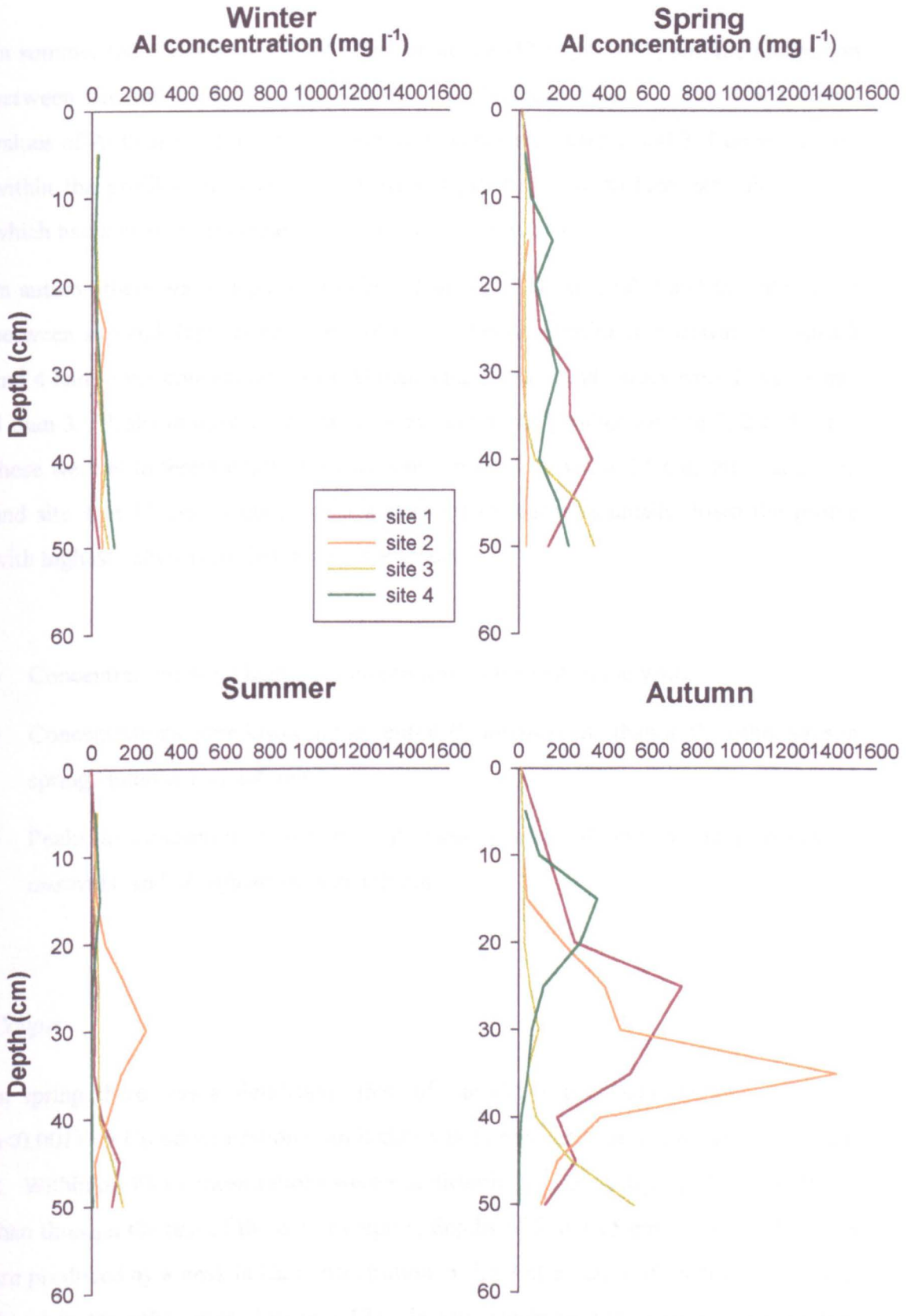


Figure 4.16. Al concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

In summer there was a significant effect of site ($F=37.1$, $p<0.001$) and the interaction between site and depth ($F=4.5$, $p<0.001$) on Al concentration. Site 4 had higher values of Al than site 1 and these were both higher than sites 2 and 3. Concentrations within the profiles increased slightly with depth from the surface excepting site 2, which had a peak in Al concentration at a depth of 30 cm.

In autumn there was a significant effect of site ($F=27.0$, $p<0.001$) and the interaction between site and depth ($F=3.9$, $p<0.001$) on Al concentration in porewaters. Sites 3 and 4 had lower concentrations of Al than sites 1 and 2 and values were lower in site 4 than 3. Peaks in concentrations were evident in the profiles for site 1, 2 and 4 but these were at different depths for each site. In site 1 it was at 25 cm, site 2 at 35 cm and site 4 at 15 cm. Concentrations in site 3 increased gradually down the profile with highest values recorded in the base 10 cm.

- Concentrations were higher in autumn than in the rest of the year.
- Concentrations were lower in the central *P. australis* site than in the other sites in spring, summer and autumn.
- Peaks in concentration occurred at approximately 30 cm in the proximal *P. australis* and *J. effusus* sites in autumn.

Copper

In spring there was a significant effect of site (11.9, $p<0.001$) and depth ($F=6.1$, $p<0.001$) on Cu concentration with higher values recorded for site 4 than sites 1 and 2. Within profiles concentrations were significantly greater at depths of 10 and 15 cm than through the rest of the core excepting depths of 5 and 15 cm. These differences are produced by a peak in Cu concentration in site 3 at a depth of 15 cm which is not found in the other sites (Figure 4.17). In the remaining site profiles concentration decreased gradually with depth, except for a small peak in concentration at 20 cm in site 1.

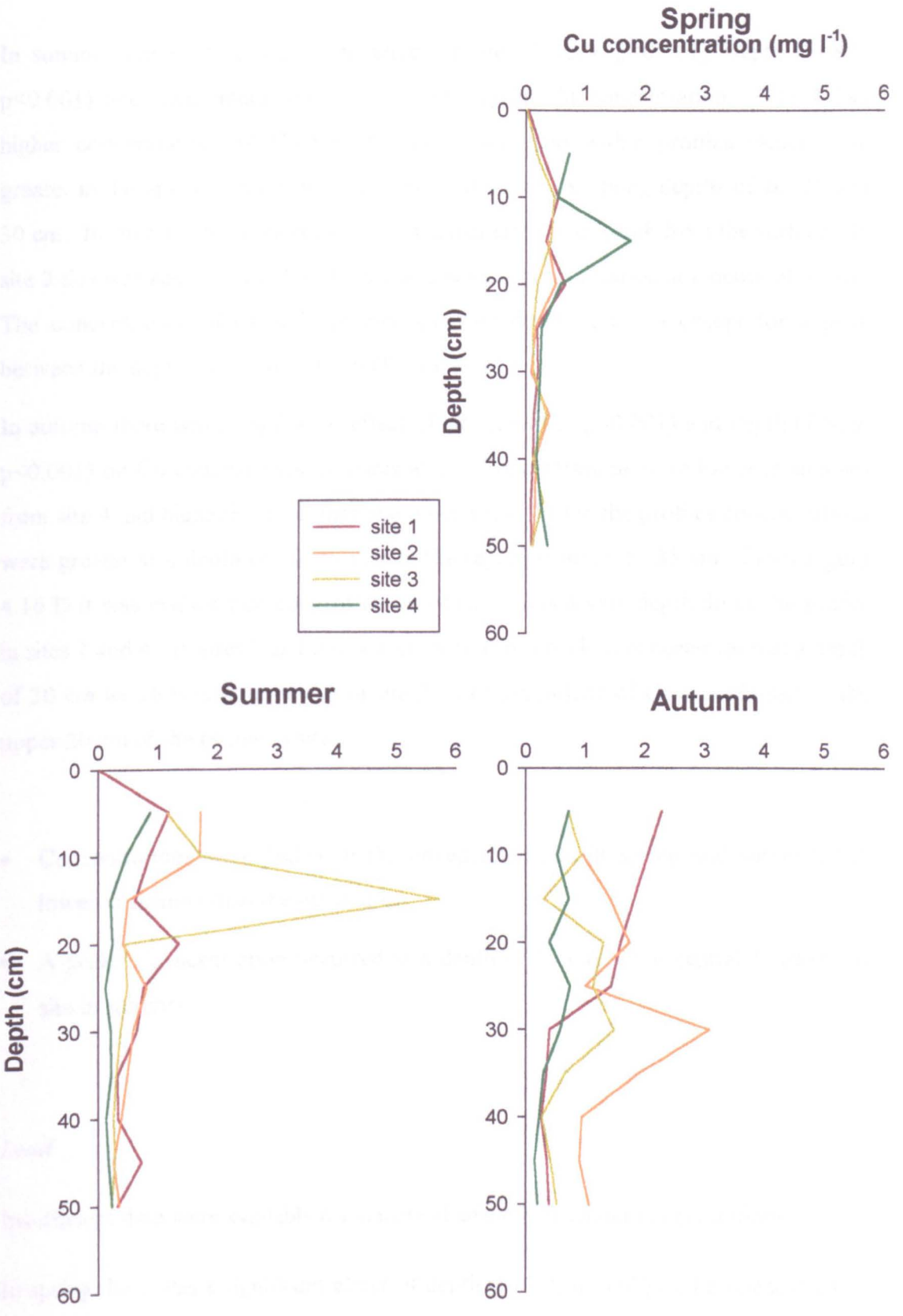


Figure 4.17. Cu concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

In summer there was a significant effect of site ($F=15.9$, $p<0.001$), depth ($F=8.9$, $p<0.001$) and their interaction ($F=2.1$, $p<0.01$) on Cu concentration. Site 4 had higher concentrations of Cu than the other sites and within profiles values were greater in the upper 15 cm than in the rest of the core excepting depths of 20, 25 and 30 cm. In sites 1 and 4 concentration of Cu increased with depth from the surface. In site 2 this was also the case but there was a peak in concentration at a depth of 30 cm. The concentration of Cu did not increase with depth in site 3 except for a peak between the depths of 20 and 30 cm (Figure 4.16)

In autumn there was a significant effect of site ($F=14.2$, $p<0.001$) and depth ($F=4.9$, $p<0.001$) on Cu concentration in porewaters. Concentrations were lower in samples from site 4 and higher in site 2 than the other sites. Within the profiles concentrations were greater at a depth of 40 cm than in the region from 20 to 35 cm. From Figure 4.16 D it was evident that concentrations of Cu declined with depth down the profile in sites 1 and 4. In sites 2 and 3 however there was a peak in concentration at a depth of 30 cm which was pronounced in site 2. Concentrations of Cu were higher in the upper 20 cm of the profile in site 1.

- Concentrations were higher in the unvegetated site in spring and summer, but lower in autumn than the other sites.
- A peak in concentration occurred at a depth of 15 cm in the central *P. australis* site in summer.

Lead

Insufficient data were available for statistical analysis of winter concentrations.

In spring there was a significant effect of depth ($F=2.3$, $p<0.05$) on Pb concentration. Within the profiles values were greater at a depth of 15 cm than in the rest of the core.

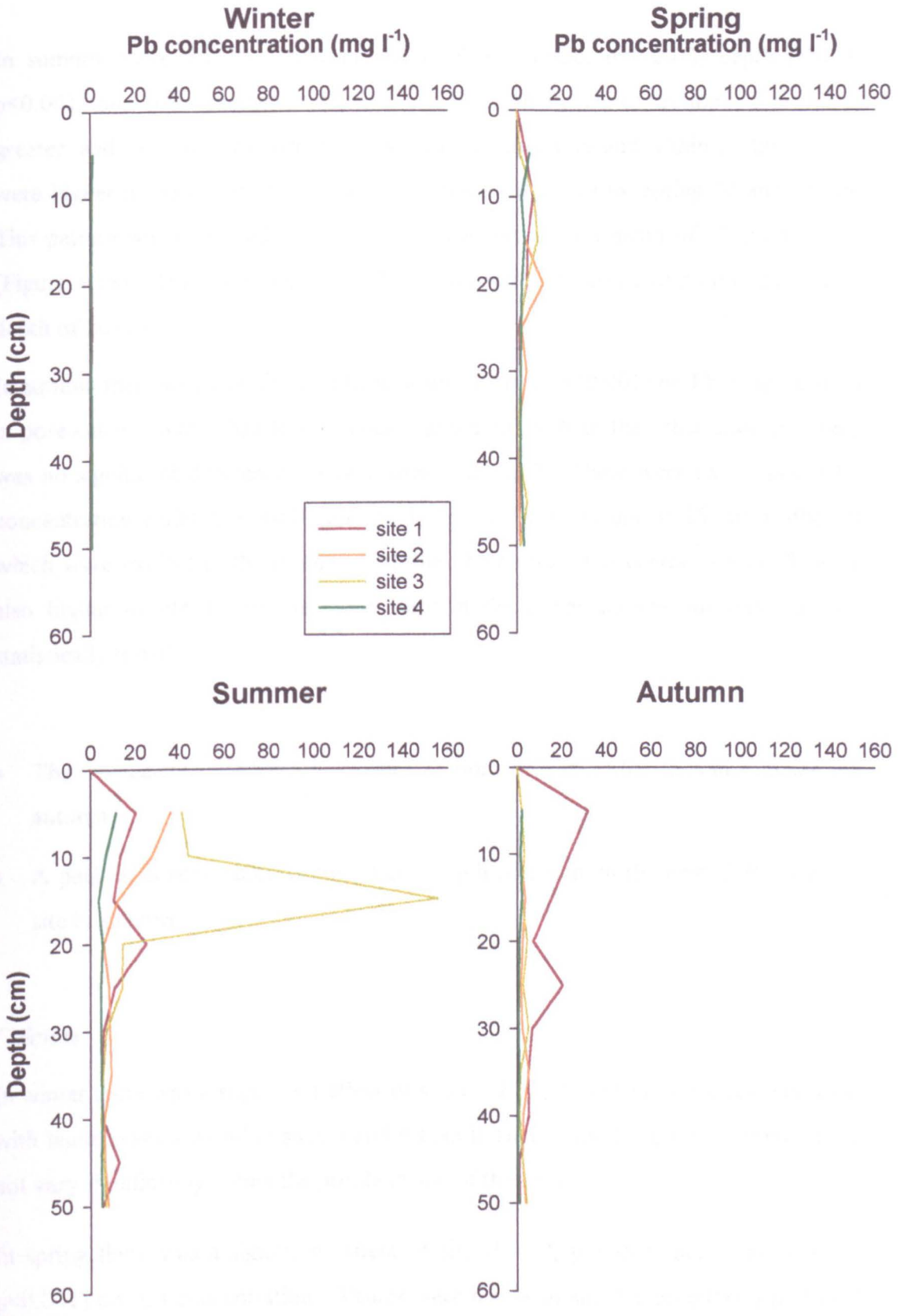


Figure 4.18. Pb concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

In summer there was a significant effect of site ($F=20$, $p<0.001$), depth ($F=8.3$, $p<0.001$) and their interaction ($F=2.9$, $p<0.001$) on Pb concentration. Site 3 had greater, and site 4 lower concentrations than the other sites and within profiles values were higher in the upper 15 cm than in the rest of the core excepting 20 and 25 cm. This pattern was produced by a peak in concentration at a depth of 15 cm in site 3 (Figure 4.18). There was also a small increase in the Pb concentration in site 1 at a depth of 20 cm.

In autumn there was a significant effect of site ($F=10.8$, $p<0.001$) on Pb concentration in porewaters. Site 4 had lower concentrations of Pb than the other sites and there was no significant difference between sites 1, 2 and 3. There were two peaks in Pb concentration within the profile for site 1, one at 5 cm and one at 25 cm, neither of which were evident in the profiles from the other sites. Concentrations of Pb were also higher in site 1 than the other sites in the upper 20 cm but this was not statistically tested.

- The unvegetated site has lower concentrations than the other sites in summer and autumn.
- A peak in concentration occurred at a depth of 15 cm in the central *P. australis* site in summer.

Calcium

In winter there was a significant effect of site ($F=21.5$, $p<0.001$) on Ca concentration with higher values found in sites 3 and 4 than in sites 1 and 2. Ca concentration did not vary significantly within the profile in any of the sites.

In spring there was a significant effect of site ($F=9.7$, $p<0.001$) and depth ($F=7.1$, $p<0.001$) on Ca concentration. Values were higher in site 3 than either site 1 or 4 and within profiles depths of 45 and 50 cm had greater concentrations than the rest of the core except for 5 and 20 cm. This pattern was produced by a dip in concentration at 20 cm in sites 4 and 1. Overall concentration increased with depth from the surface (Figure 4.19).

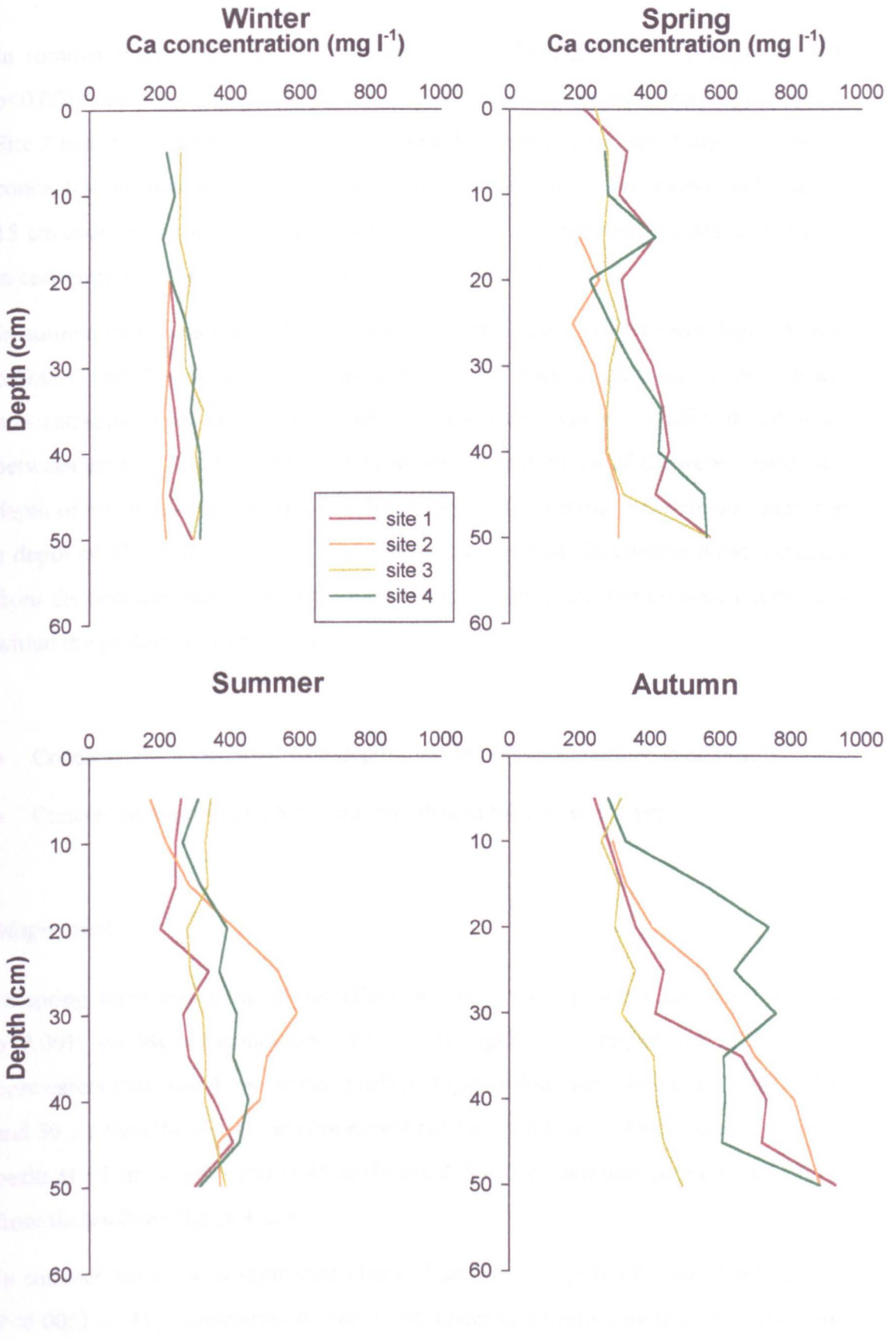


Figure 4.19. Ca concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

In summer there was a significant effect of site ($F=11.2$, $p<0.001$), depth ($F=6.1$, $p<0.001$) and their interaction ($F=2.9$, $p<0.001$) on Ca concentration in porewaters. Site 2 had significantly higher concentrations than 1 and 3, and site 4 also had greater concentrations than site 1. Within the profiles concentrations were lower in the upper 15 cm than the within the range of 30 to 45 cm. This effect was produced by a peak in concentration of Ca in the zone in site 2 (Figure 4.19).

In autumn there was a significant effect of site ($F=16.6$, $p<0.001$) and depth ($F=6.4$, $p<0.001$) on Ca concentration in porewaters. Site 3 had significantly lower concentrations than any of the other sites but there was no significant difference between sites 1, 2 and 4. Within the profiles concentrations of Ca were greater at a depth of 50 cm than in the region of 20 to 35 cm. Concentrations were also higher at a depth of 45 cm than 20 cm. Figure 4.19 showed that Ca concentration increased from the sediment surface down the profile for all sites, but the greatest fluctuations within the profile occurred in site 4.

- Concentrations increased with depth from the sediment surface in all profiles.
- Concentrations were higher in autumn than in the rest of the year.

Magnesium

In spring there was a significant effect of site ($F=6.6$, $p<0.01$) and depth ($F=8.4$, $p<0.001$) on Mg concentration. Site 1 had significantly higher concentrations in porewaters than site 4 and within profiles higher values were found at depths of 45 and 50 cm than the rest of the core except for 15 and 40 cm. This is caused by small peaks at 15 cm in site 4 and at 45 cm in site 2, but concentration increases with depth from the surface (Figure 4.20).

In summer there was a significant effect of site ($F=5.1$, $p<0.005$) and depth ($F=3.3$, $P<0.005$) on Mg concentration. Site 1 had lower concentrations than the other sites and within profiles values were higher at a depth of 40 cm than at 5 and 15 cm.

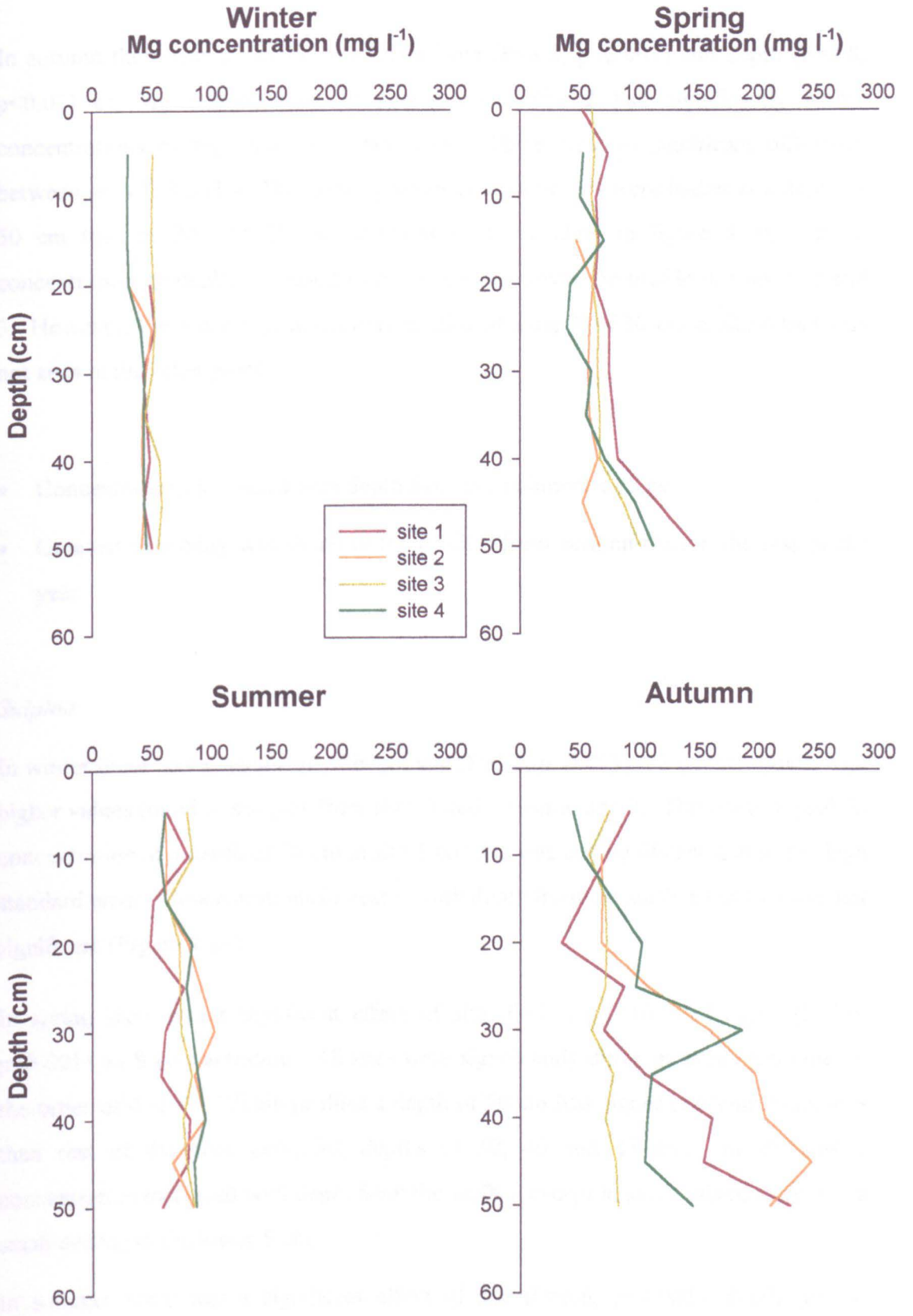


Figure 4.20. Mg concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

In autumn there was a significant effect of site ($F=8.8$, $p<0.001$) and depth ($F=3.8$, $p<0.01$) on Mg concentration in porewaters. Site 2 had significantly higher concentrations of Mg than the other sites. There was no significant difference between sites 1, 3 and 4. Within the profiles concentrations were higher at a depth of 50 cm than at 20 and 25 cm which was also evident in figure 4.20, where concentration gradually increased from the surface down the profile in sites 1, 2 and 3. However, there was a peak in concentration at a depth of 30 cm in site 4 that was not seen in the other profiles.

- Concentrations increased with depth from the sediment surface.
- Greatest variability was found in the profiles from autumn than in the rest of the year.

Sulphur

In winter there was a significant effect of site ($F=3.3$, $p<0.05$) on S concentration with higher values found in samples from sites 3 and 4 than in site 2. There was a peak in concentration at a depth of 30 cm in site 1 but this was not significant due to the high standard error. Concentrations increased with depth from the surface but this was not significant (Figure 4.21).

In spring there was a significant effect of site ($F=7.4$, $p<0.01$) and depth ($F=6.6$, $p<0.001$) on S concentration. All sites were significantly different from each other in the order of 4>1>3. Within profiles a depth of 50 cm had higher concentrations of S than rest of the core excepting depths of 30, 40 and 45 cm. In all sites S concentration increased with depth from the surface except in site 1 where there was a small decline in the lower 5 cm.

In summer there was a significant effect of site ($F=6.6$, $p<0.001$), depth ($F=5.7$, $p<0.001$) and their interaction ($F=4$, $p<0.001$) on S concentration in porewater with lower concentrations found in site 1 than the other sites. Within the profiles significantly higher concentrations were found at depths of 30, 45 and 50 cm than in the upper 20 cm. Concentrations of S gradually increased down the profile in all sites

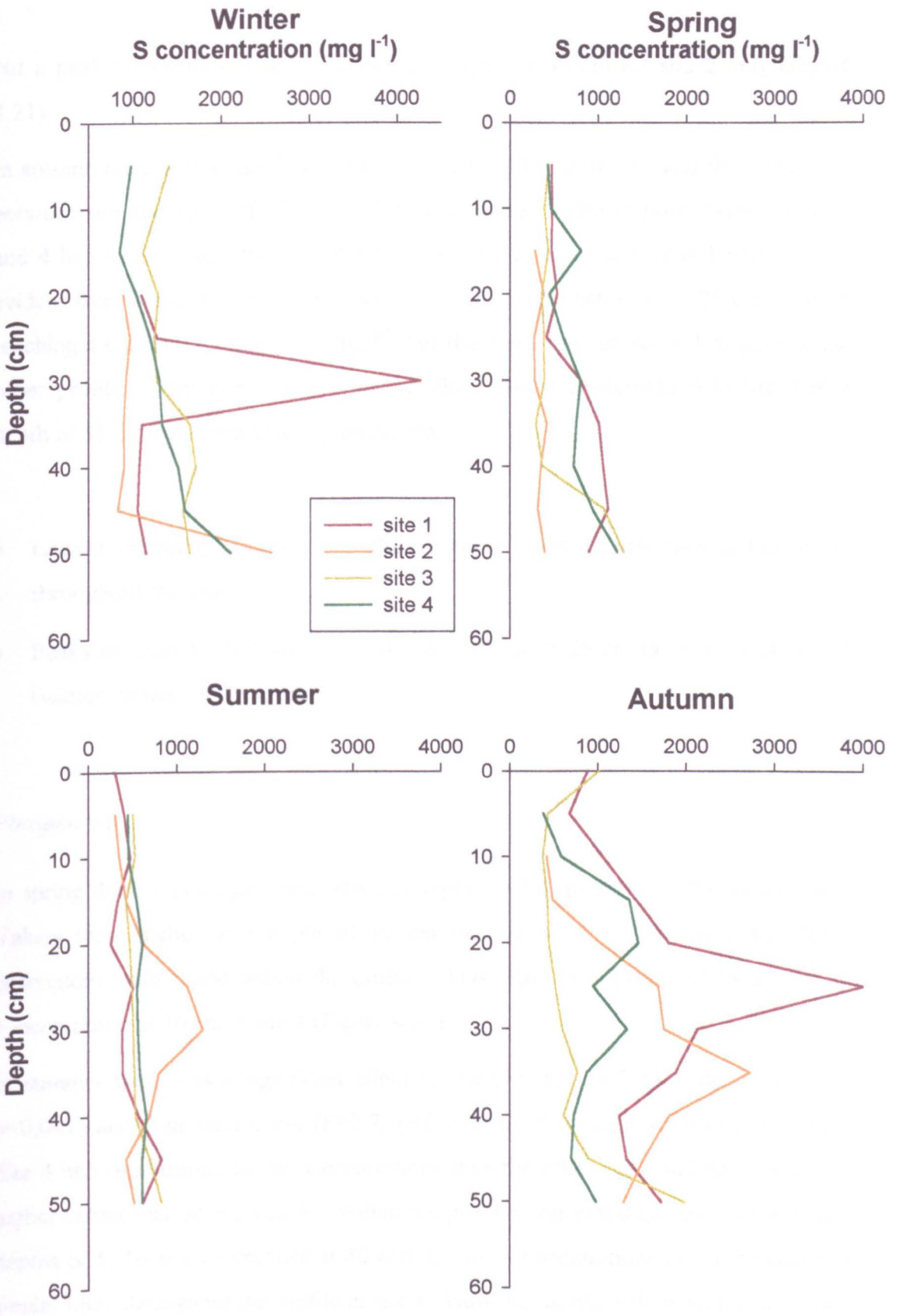


Figure 4.21. S concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

but a peak in concentration was found at a depth of 30 cm for site 2 only (Figure 4.21).

In autumn there was a significant effect of site ($F=19.9$, $p<0.001$) and the interaction between site and depth ($F=2.4$, $p<0.01$) on S concentration in porewaters. Sites 3 and 4 had lower concentrations of S in porewater samples than sites 1 and 2. It is evident from figure 4.21 that there was a peak of S concentration at 25 cm in site 1 reaching a concentration of 4000 mg l^{-1}, but that there was no equivalent peak in the other profiles. However, there was a smaller peak of concentration in site 2 at a depth of 35 cm but this was less pronounced.

- Lowest variability was found in the central *P. australis* site than in the others throughout the year.
- Peaks in concentration were recorded at a depth of 25 cm (autumn) and 30 cm (winter) in the *J. effusus* site.

Phosphorus

In spring there was a significant effect of depth ($F=2.1$, $p<0.05$) on P concentration. Values were higher at a depth of 10 cm than at 35 cm. No other significant differences were found within the profile. This effect was produced by a peak in concentration at 10 cm in site 4 (Figure 4.22).

In summer there was a significant effect of site ($F=18.9$, $p<0.001$), depth ($F=18.6$, $p<0.001$) and their interaction ($F=3.7$, $p<0.001$) on P concentration in porewaters. Site 4 had significantly lower concentrations than the other sites, and site 3 also had higher values than sites 2 and 4. Within the profiles concentrations were greater at a depths of 5, 10 and 15 cm than at 40 and 45 cm. Concentrations of P remained at a similar level throughout the profile in site 4. However in site 3 there was a significant peak in concentration at a depth of 15 cm.

In autumn there was a significant effect of site ($F=3$, $p<0.05$) on P concentration in porewaters. Site 1 had higher concentrations of P than site 4, but there was no

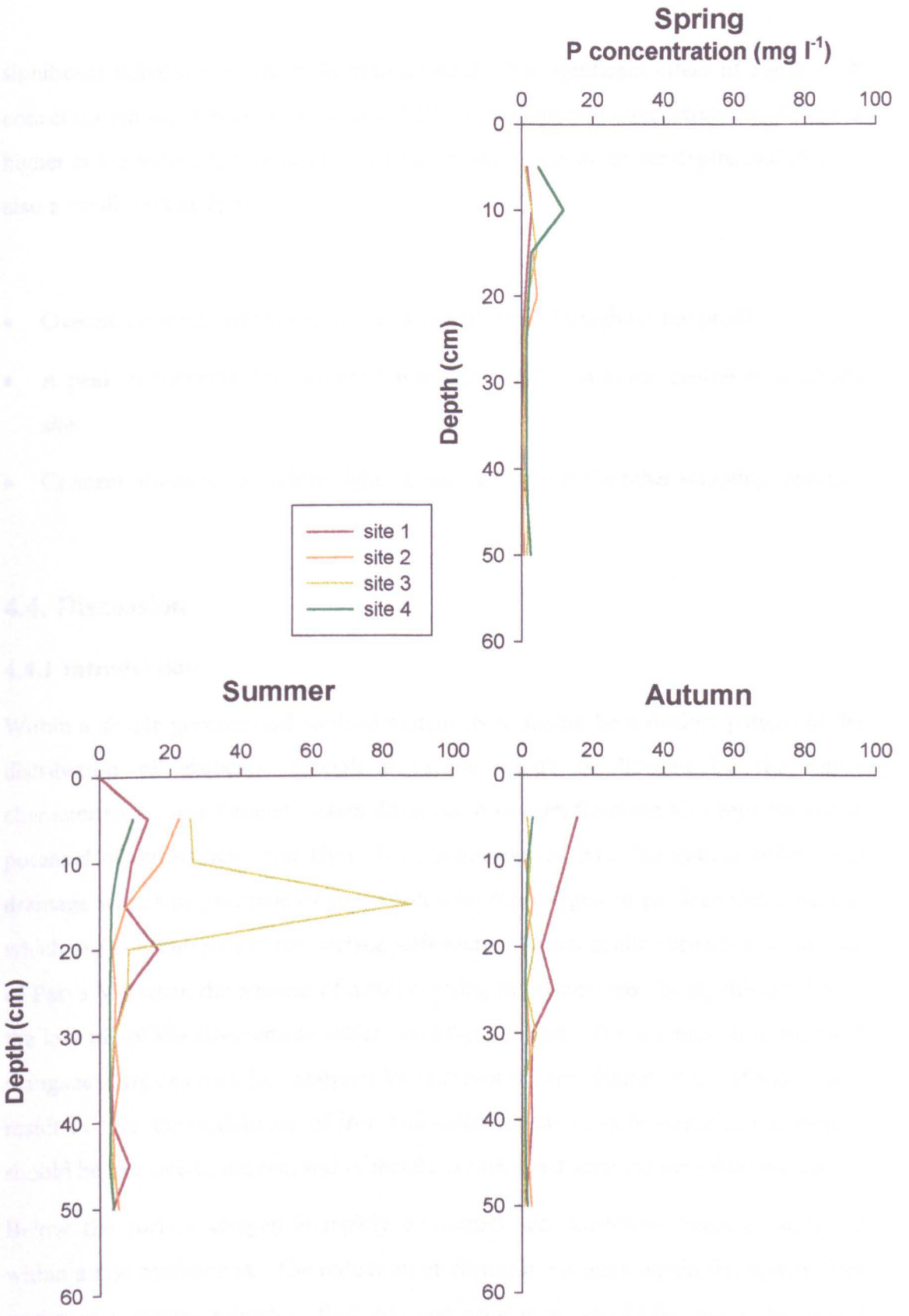


Figure 4.22. P concentration profiles at sampling sites (1) *J. effusus* pool, (2) *P. australis* edge, (3) *P. australis* centre and (4) unvegetated from Mam Tor.

significant difference between the sites 2 and 3. No significant effect of depth on P concentration was found. From figure 4.22 it is evident that concentrations of P were higher at the sediment surface (5 to 10 cm) in site 1 than at lower depths and there is also a small peak at 25 cm.

- Overall, concentrations remained at a similar level throughout the profiles.
- A peak in concentration occurred at a depth of 15 cm in the central *P. australis* site.
- Concentrations were slightly higher in summer than in the other sampling months.

4.4. Discussion

4.4.1 Introduction

Within a simple geochemical wetland system there should be a distinct pattern in the distribution of elements through a profile which is dictated by the redox characteristics. In a flooded system diffusion of oxygen from the air keeps the redox potential of the surface layer high. Metals transported into this system either from drainage waters or precipitation may react with this oxygen to produce metal oxides, which are concentrated in the surface sediments. Within acidic systems such as that of Parys Mountain the amount of metal entering the system may be significant due to the low pH of the environment which solubilises metals. The production of iron and manganese oxides may be catalysed by micro-organisms (Batal *et al.* 1989). As a result of this, concentrations of iron and other metals in porewaters at the surface should be low due to the removal of metals as they react with the available oxygen.

Below the surface oxygen is rapidly consumed and conditions become anaerobic within a few centimetres. The reduction of chemical elements within the system then occurs in a specific sequence, thus iron and manganese should become reduced and porewater concentrations increase below the oxygenated layer. Sulphate reduction would also be initiated which is facilitated by the activity of bacteria (figure 4.23)

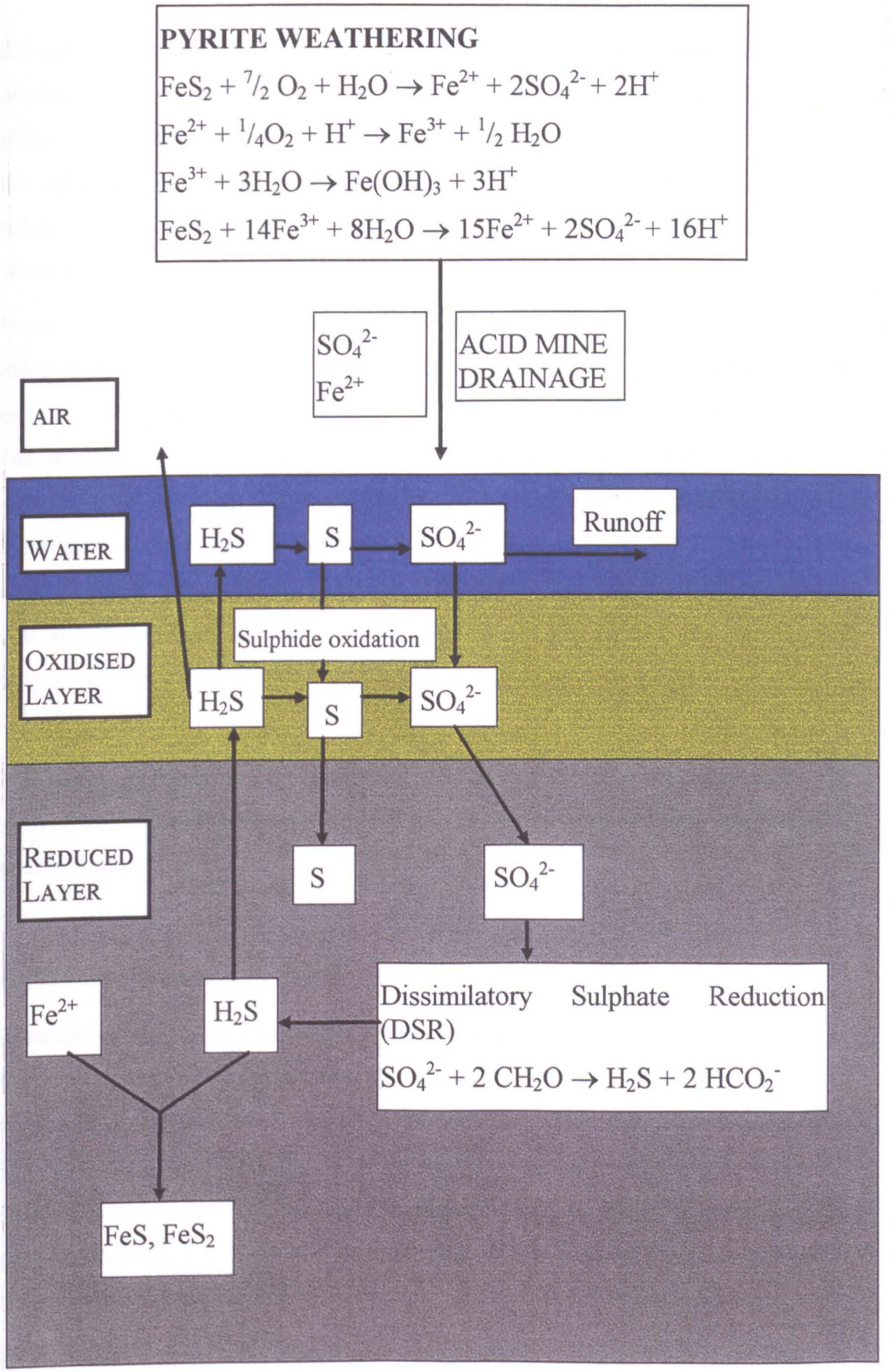


Figure 4.23 Sulphur cycling within a wetland system receiving AMD.

through a process known as dissimilatory sulphate reduction. The main species involved in this process have been identified as *Desulfovibria* and *Desulfotomaculum* (Connell & Patrick 1968; Postgate 1979). This produces H_2S, which may react with the reduced iron together with other reduced metals including Zn and Cu to form sulphide minerals. This would remove metal elements and sulphur species from the water column.

However, the processes of metal transformation are not as straightforward as this and other factors may be involved in the distribution of metals within wetlands. For example, Donahoe & Liu (1998) found that the concentration of Ca, Mg, Na, Fe and Mn were higher in the zone of *Juncus effusus* than in open water or *Nymphaea odorata* areas indicating that vegetation in general and the specific plant species may affect distribution of metals. Concentration peaks have also been reported to occur in areas of higher rooting density (Caçador *et al.* 1996). In Chapter 3 it was suggested that metal uptake may also vary between plant species and this may in turn affect the concentrations of metals in the porewaters surrounding the root systems.

The results from the two study sites reflect this complexity in natural systems with a high degree of variability both within and between distribution profiles throughout the year. However, some main features and patterns can be identified and some possible causes proposed.

4.4.2. Parys Mountain

Throughout the year concentration of iron tended to be higher in the porewaters of the vegetated zones than in the unvegetated stream site within the profile. This may be due to the higher retention times of contaminated water in the vegetated areas as flow rate is slowed. This allows greater levels of metals to accumulate within the sediment and its associated porewaters. However, this is not the case for all metal profiles indicating that there may be other processes controlling the amount of metal that is retained in the porewaters, and that these may differ with metal species.

Fe may also be higher in vegetated than unvegetated sites due to the oxidation activity of roots. The release of O_2 via R.O.L. into the sediment could cause the oxidation of previously formed iron sulphides thereby releasing Fe(II) into the porewaters. This

has previously been observed in wetlands where an increase in porewater Fe was suggested to indicate that Fe sulphides were oxidised and a net loss of pyrite was observed from April to June (Kostka & Luther 1995). However, in environments with high dissolved metal loads, other metal ions may react with sulphide to form a variety of sulphide minerals. In these more chemically complex systems Cu and Zn form sulphides more readily than Fe (Machemer & Wildeman 1992). It would therefore be expected that these metals would also be released into porewaters during the same period of oxidation and although there is some difference in concentrations of Zn and Cu in the summer months this does not reflect the pattern seen for Fe. Sulphur concentration would also be expected to increase in porewaters upon oxidation of sulphide minerals but this is not seen in the profiles. It seems unlikely therefore that the formation of metal sulphides and their subsequent oxidation is the major control on the distribution of the metals. This does not agree with the findings of Johnson *et al.* (1993) who reported the presence of a zone of ferrous sulphide formation at 8-15 cm from the sediment surface at Parys Mountain, however the time of year during which the study was undertaken was not stated and it may be that reduction and oxidation processes are more important in other times of the year. Indeed, in the winter months when plant activity ceases there is a dip in both porewater Fe and S at a depth of 10-20 cm in the *E. angustifolium* and unvegetated zones indicating the possible formation of Fe sulphides at this depth. This would not be the case in the zones of *P. australis* growth as flow of oxygen continues through the dead culms during the winter months causing oxidation of the substrate to continue. Overall concentrations of Fe and S are higher in the porewaters during this period but this is probably due to an increase in supply from increased runoff during the winter months. Concentrations of Zn and Cu do not show this dip in concentration to the same degree but are lower throughout the profiles in the winter than autumn which could reflect their higher reactivity with sulphide. Cu and Zn retention has been shown to be largely controlled by sulphide precipitation in wetlands (Griffin *et al.* 1989). Concentrations of Al are similar between the summer and winter months suggesting that sulphide formation is not important which is expected as Al does not form sulphide minerals. However Al may form $AlSO_4$ minerals when pH is low and Al concentration high (Howarth & Stewart 1992; Nordstrom 1982) therefore Al concentrations should be lower in winter when SO_4^{2-} supply is increased. In

summer evaporative effects could concentrate Al in porewaters thereby increasing its reactivity to sulphate ions and thus reducing the amount of Al in the interstitial waters, or it may simply reflect a decrease in supply in summer months.

In spring, summer and autumn, concentrations of Fe are lower at the sediment/water interface (5 cm depth) than at lower levels in the profiles, particularly in the vegetated zones. This is in agreement with the previous model outlined where the oxidised layer enables the production of iron oxides thereby removing iron from the interstitial waters. This can be seen visually in the cores as an orange/yellow layer at the surface of the sediment which extends approximately 5 to 10 cm into the sediment core, but this varies through the year. Parkman *et al.* (1996) also reported the formation of orange-brown ochres in the stream sediments of Parys Mountain and these were identified as x-ray amorphous or very poorly crystalline iron oxides.

The formation of iron oxides in the surface layers may also affect the concentrations of other elements. Hydrous Fe oxides have a high specific surface area and possess OH functional groups capable of reacting with metals and other cations and anions (Kuo 1986). The adsorption of Ca, Cd, Cr, Cu, Pb, Zn and phosphate onto iron oxides have all previously been reported (Kuo & McNeal 1984; Ghanem & Mikkelson 1988; Coston *et al.* 1995; Geelhoed *et al.* 1997; Lin & Chen 1998). In addition analysis of sediment fractions have shown that Cd, Pb, Ni, Cu and Zn were positively correlated to the amount of hydrous Fe and Mn oxides present (Feijtel *et al.* 1988). However, the results from Parys Mountain show that the pattern of distribution of the other metals does not reflect this and in the case of Al and Pb the concentrations may be higher in the porewaters of this zone than in the rest of the profile. Concentrations of Cu and Zn however, are lower in the surface region than at lower depths which could indicate the adsorption of these metals. In fact Zn is preferentially adsorbed by iron oxides over Pb (Coston *et al.* 1995) and Johnson (1986) also reported the high affinity of iron oxides for Zn and Cu. Bowell & Bruce (1995) also suggested that at pH's of less than 5.0, As, Sb, Mo and Zn are strongly adsorbed and Pb and Mn are not adsorbed onto iron oxides until pH is at a higher level. Cu and Zn could therefore be effectively competing for adsorption sites so preventing the depletion of the other metals in this zone.

The depletion of Fe in the upper layers is not seen in the non-vegetated site which may be due to evaporation effects during the warmer months which would be greater in this exposed site. The evaporation would aid the movement of porewater and thus dissolved iron up the profile and produce a more even distribution of Fe through the core. This has previously been reported for the movement of dissolved sulphide in wetlands (Bottrell & Novak 1997) and is also evident in the porewater profiles of S in Parys Mountain (Figure 4.11). However, the presence of organic matter could also be an important factor affecting these patterns of Fe distribution. Highest metal concentrations associated with sediments were found in the surface layer (0-1 cm) of wetlands and increased with depth. Most of these metals were bound to the organic matter (Tam & Wong 1996). In addition complexation of Cu and perhaps other metals by organic matter associated with ochreous precipitates has bee indicated (Winland *et al.* 1991). This could also cause the depletion of Fe, Cu and Zn seen in the profiles from Parys Mountain.

At lower depths in the profiles oxygen becomes depleted, iron will become reduced and thus solubility will increase resulting in higher concentrations in interstitial waters. Manganese should also be reduced and this is evident in the core profiles where the concentration of Mn increases at lower depths, however this occurs nearer to the sediment surface than the Fe reduction zone in both spring and summer. Mn is reduced earlier in the redox reaction series (Table 1.1). and it has been suggested that Fe(III) is not reduced until microbially reducible Mn(IV) is depleted (Hamman & Ottow 1974; Ottow 1977; Munch & Ottow (1983). The region where Mn is reduced would therefore occur just above that where iron reduction occurs (Myers & Nealson 1988). Lovley & Phillips (1988) reported that Fe(II) from the iron reduction zone diffusing up into the overlying zones of Mn(IV) reduction is consumed anaerobically and this would add to the Fe depletion seen in the upper layers. This pattern however is not evident in the profiles from the autumn where there are very low concentrations of Fe in the porewaters throughout the core. One possible explanation for this is that rainfall increases in the autumn in this area which could lead to a flushing through of the system, diluting the porewaters.

In addition to these processes the presence of organic matter can also influence the distribution of metals. Adsorption of metals onto organic matter can be a significant

removal process in wetlands and it has been shown to be the most important mechanism of Fe retention up to the saturation point of organic sites (Henrot & Wieder 1990). During the autumn when dieback of plant species occurs there will be a significant input of organic material into the upper layers of the sediment and therefore adsorption of metals onto these sites would increase. This process will be more important for Fe and Cu than Zn and Mn as they compete for adsorption sites on organic matter (Henrot & Wieder 1990; Machemer & Wildeman 1992). Organic compounds may also be an important sink for sulphate in the surface 10 cm of peat (Brown & McQueen 1985) however, concentrations are not significantly lower in the vegetated than unvegetated zones and so it unlikely that this is an important process in this wetland. In addition Cu has an extremely high affinity for organic matter (Shotyk 1988) and concentrations actually increase in the porewaters of Parys Mountain in the *E. angustifolium* zone in autumn again suggesting that this is not a significant process. Removal efficiency of wetlands with regards to Cu has been shown to decrease in autumn months and this was thought to be caused by either an increase in high strength mine drainage or the effects of cold weather on biological activity (Sobolewski 1996). The influx of organic matter and carbon in autumn is equally as likely to cause an increase in metal concentration as it would encourage the activity of oxidising bacteria which could oxidise previously formed sulphides and release metals into the porewaters. In fact, organic matter influx into the wetland may not be of major importance in these wetlands as decomposition of macrophyte litter has been proved to be impeded at low pH (Kittle *et al.* 1995).

Adsorption of metals onto organic matter may still occur however, and this is an important consideration at Parys Mountain due to the nature of the sediments. The cores taken showed a high degree of variability in the sediment characteristics through the profile with three distinct layers. In the upper 10 cm there is a layer of orange/yellow ochre which is unconsolidated. From 10 to 45 cm the sediment is composed of a black/purple coloured peaty layer, and in the bottom 5 cm of the core is an orange gravel layer. The peaty layer may adsorb more metals than the other layers and indeed in many of the profiles there is an increase in metal concentration in the lowest 5 cm of the profile. The sediment characteristics also vary with site, the *E.*

angustifolium zones have a thinner layer of iron oxides at the surface and a thicker layer of peat but metal concentrations are not always lower in these areas.

Although some patterns in the distribution of metals have been identified it is not clear which of the many processes that control the occurrence of metal species are the most important. It may be that different processes are more significant at certain times of year, in different areas within the wetland and at different depths within the sediments.

4.4.3. Mam Tor

The movement of water through the wetland system at Mam Tor is very different from that at Parys mountain. The wetland is situated on the toe of the landslide and is supplied with acidic sulphate waters from the crush zone within the slide diluted with meteoric waters (Vear & Curtis 1981). Replenishment of groundwater during winter is unusual in that, although it is partly derived from vertical infiltration it is chiefly brought about by throughflow of groundwater within the slide debris from higher parts of the landslide (Skempton *et al.* 1989). This would result in a supply of sulphate and dissolved metals from the groundwater rather than from surface streamflow as at Parys mountain.

The profiles for Mn, Mg, Ca and Al all show a very similar pattern with low concentrations in winter, spring and summer and an increase in concentrations in autumn. This is presumably due to replenishment by increased rainfall and movement within the landslide during the autumn which flushes in a high concentration of these elements. These elements would then be removed during the winter months through a variety of processes. In contrast to Parys mountain the sedimentary characteristics of the profiles from Mam Tor do not vary as much through the core, with the majority made up of a black peat. This would result in a similar adsorption capacity throughout the profile. In the upper layers (5-15 cm) many of the cores have a distinctive orange/red colour to the sediment similar to that seen at Parys Mountain, but at Mam Tor there is also a significant amount of *Sphagnum* in these upper layers. The formation of Mn and Fe oxides in the surface layers could also affect the other metals, in particular Mn oxides are efficient scavengers of other elements including Ca and Mg (Donahoe & Liu 1998). This is reflected in the profiles for Mg, Ca, Al, Mn,

Zn and Cu which all show lower concentrations in the upper layers in spring and summer.

In autumn, input of contaminated drainage into the system will increase and the redox boundary will move closer to the surface of the peat as the water table rises thereby allowing reducing conditions to occur through a greater part of the profile which would then release Mn and Fe into the porewaters. This would also cause an influx of other elements that had been adsorbed to the surfaces of the metal oxides into the interstitial waters. This is evident in the majority of the metal profiles particularly in site 1, however Fe does not show this pattern. In Parys Mountain it was shown that Mn is reduced before Fe and therefore the release of Mn should occur nearer the surface than Fe. Fe is released at a lower depth in autumn of about 10 to 20 cm depending on site, which is lower down the profile than the Mn release so supporting this theory. During the winter months however, continuous reducing conditions may allow the formation of sulphides which would remove metals from porewaters which is evident in the profiles for Mn, Cu, Zn, Fe and Pb. If metal sulphides were forming then sulphur concentrations would also be expected to decrease from autumn to winter which is the case excepting for an anomaly at 30 cm in site 1. Fluctuations within the profiles also occur for some of the other elements including iron which also shows a peak in concentration from 20 to 30 cm depth in the autumn at site 1. Damman (1978) reported the occurrence of a local maximum in Fe concentration approximately 20 cm below the bog surface which corresponded to the maximum height of the water table. The peak evident at Mam Tor could therefore reflect the fluctuation in height of the water table during the year. However, if this were the case then the other elements, particularly Mn should also reflect this movement in water table and this is not the case. If sulphide formation is to be an important consideration in wetlands then a sufficient supply of organic matter is required (Kleinmann 1990) which is likely at Mam Tor where there is significant growth of various species including *Sphagnum*. Site 1 is colonised by the species *J. effusus* and the rooting zone occurs at a depth of 20-25 cm corresponding to the peaks in Fe, S and Zn in the autumn. Dieback of this species at the end of the growing season would release C into the surrounding media which is a focus for reducing bacteria. Therefore this would encourage the reduction of previously formed oxides which may have formed

around the roots of the wetland species and so release iron and associated metals into the surrounding waters. This would not occur at site 3 where *P. australis* is the dominant species as oxygen continues to be released into the subsurface layers due to venturi-flow through the dead culms.

Al shows the same overall pattern as at Parys Mountain, with lower concentrations occurring in the winter and summer months than in the rest of the year. Again this could be due to the formation of Al sulphate minerals in winter as sulphate input to the system increases.

4.5 Conclusions

It has been shown that the distribution of metals within wetland systems can be quantified using chemical analysis of porewaters. However, it is clear from the data that these systems are chemically dynamic and a large number of different chemical and biological processes may affect the metal distribution. It has been suggested that in the winter months the formation of Cu, Zn and Fe sulphides can reduce the concentrations of these elements within porewaters, but that in the surface layers, the oxidation of Fe and Mn is important in removing elements from porewaters both in the winter and summer. The formation of sulphide minerals is achieved in reducing conditions but the same reducing conditions may also act to increase concentrations of elements in interstitial waters by increasing their solubility. It has been proposed that this occurs in the spring and summer months when sulphate input is low so inhibiting the formation of sulphide minerals. It has also been suggested that the water table may be an important control on distribution of metals as it can affect the redox boundary within the system. In different wetland systems the water table may show variable fluctuation which is dependent upon the dominant flow of water through the system. At Mam Tor contaminated water moves along the base of the slide and thus upwards through the wetland profile, whereas the majority of input water at Parys Mountain originates from surface flow therefore the dominant movement is down through the profile. As a result the water table at Mam Tor may show greater variability and be a more significant control on chemical processes.

Thus it is extremely difficult to separate out the different processes that may affect the distribution of metals from the chemical evidence of the porewaters alone. In such dynamic systems each removal process may be either inhibited or catalysed by another, may be more significant at different times of the year and may be of varying significance depending on location. It was hypothesised that plant root activity may be important in the removal of metals in these systems but it is not possible to determine this from the field data collected.

5. Metal removal processes in wetland microcosms.

5.1 Introduction

It has been shown in chapter 4 that within natural systems a number of different processes operate which affect the distribution of metals both horizontally and vertically within the wetlands. However it was also suggested that natural systems are too complex to enable the identification of the individual processes and in particular to identify the effect of plant growth and rhizospheric activities. Specifically, at Parys mountain the nature of the sediment is highly variable within the system and this is a major factor affecting metal distribution. Sediments can vary in a number of ways, each of which can change the adsorption capacity and thus the amount and type of metals that are associated with the different fractions. This alters the balance of the system and either removes or makes available metals for other redox-related reactions. It has been shown that metal adsorption differs with the texture of the sediment, Chambers & Sidle (1991) found that fine-textured soils had a higher concentration of Cd, Zn, Cu and Pb than coarser soils and it has also been reported that the extent of adsorption decreases with increasing adsorbent particle size (Jain & Ram 1997). The chemical nature of the sediment may also affect adsorption, fine kaolinites have been shown to have a greater affinity for Ni when compared with other clay minerals (Tiller *et al.* 1984). In addition the amount of organic matter may be important. A correlation has been found between adsorption capacity of sediments for heavy metals and organic matter content (Lin & Chen 1998), and the adsorption of Al and Fe has been proved to be significant but this was less so for Mn (Wieder & Lang 1986).

In natural systems it is also impossible to control the amount of metals entering the system which can vary over short spaces of time, and also the origin of these input waters. It was shown in chapter 4 that at Mam Tor the majority of water entered the system from the base of the slide therefore the dominant movement through the profiles was from the base upwards. In contrast at Parys mountain the main flow of

water into the wetland was from the surface drainage system and therefore movement was either from the surface downwards or horizontally through the wetland. This is an important factor in controlling the level of the water table which in turn controls redox reactions, and in flushing metals through the system at certain depths. Variations make it difficult to achieve a simple vertical profile of metal distribution controlled by chemical reactions.

In order to investigate the role of plant growth and rhizospheric processes in a wetland system therefore it is important that either the nature of the sediment is taken into account or it is homogenised. This can be achieved in the laboratory through the use of microcosm systems which also enables the standardisation of inputs and outputs of the wetland system.

The aim of this chapter was therefore to determine potential effects of the root activities of a wetland plant species on the vertical distribution of metals in a wetland microcosm.

5.2 Materials and Methods

Each microcosm was formed in a length of plastic drainpipe, 15 cm in diameter and 50 cm in length. A total of 20 microcosms were prepared allowing for 5 replicates each of 4 treatments. The resulting cylinders were then soaked in Decon for 24 hrs, rinsed in UHP 3 times and then dried thoroughly. A circle of 1 cm plastic mesh was glued to the base of each section using Silfix HM High modulus silicone sealant and left to dry for 24 hrs. This allowed the retention of peat within the cylinder but also enabled the movement of water through the system.

Each cylinder was filled to a depth of 25 cm with a standard peat mixture (Table 5.1) and above this a layer of muslin fabric was secured which would restrict root growth to the upper part of the pipe section. Additional peat was placed on top of this to within 2.5 cm of the top of the tube. Each tube was supported in a 30 cm diameter plastic plant tray to allow retention of the waters and continued saturation of the soil

The drainpipes were then stood in UHP water for 3 days to allow complete saturation of the soil to be attained.

	Al	Cu	Fe	Mn	Zn
Concentration ($mg\ kg^{-1}$)	48.6 ±2.43	0.30 ±.06	298.75 ±14.39	2.62 ±.08	1.11 ±.26

Table 5.1. Concentration of selected heavy metals in the peat used in the microcosm study. Means ± SE, n=3.

P.australis seedlings were grown for 28d in hydroponic cultures containing 10% Rorison's reduced to pH 3.5 through the addition of 0.1M H_2SO_4. Iron plaque was induced on their roots through the addition of 50 mg l^{-1} of Fe supplied as ammonium ferrous sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$)) for a period of 1 week. Prior to plaquing the seedling roots were soaked in acidic UHP for 12 hrs to prevent the interference of phosphate in the nutrient solution with the iron. One seedling was planted in each of ten of the drainpipe sections after saturation had been achieved. The remaining tubes were left unvegetated. 1 L of UHP water reduced to pH 3.5 using 0.1M H_2SO_4, was added from the top to each drainpipe, which were then left to acclimatise for 7d.

Artificial acid mine drainage (AMD) was prepared according to that outlined in Table 5.2 and this was based on the chemical composition of drainage waters from Parys mountain (Chapter 4).

	Concentration ($mg\ l^{-1}$)	Compound used
Al	50	$Al_2(SO_4)_3.\ 16H_2O$
Cu	10	$CuSO_4.5H_2O$
Mn	10	$MnSO_4.\ 4H_2O$
Zn	30	$ZnSO_4.\ 7H_2O$

Table 5.2. Composition of artificial AMD.

At the end of the acclimatisation period AMD treatment was started, with 1L of AMD added to 5 of the vegetated and 5 of the unvegetated systems. This resulted in 4 treatments as follows (1) vegetated (2) vegetated with AMD, (3) unvegetated and (4) unvegetated with AMD. The AMD was added from the top to allow vertical movement of the contaminated water through the whole cylinder. 1L of artificial AMD was added every 7d for a total period of 42d at the end of which each core was sectioned into 5 cm layers. The soil from each layer was placed in a clean polythene bag, sealed and stored at 4°C until porewater extraction (chapter 2.3.11).

Statistical analysis

A General Linear Model was used for two-way analysis of variance which allowed for unequal replication. This was followed by a multiple comparison Tukey test. Where data did not fit the assumptions of the GLM, the data was log transformed either by $\log_e$ or $\log_{10}$. Specifics of all statistical analyses are tabulated in appendix F. In all cases the coefficient of variance did not exceed 1.5%.

5.3. Results

Within the vegetated cylinders the rooting layer was noted to occur at a depth between 10 and 25 cm.

5.3.1 Iron

There was a significant effect of vegetation ($F=24.5$, $p<0.001$) and depth ($F=2.7$, $p<0.01$) on Fe concentrations with higher values in porewaters from the unvegetated treatment than the others. Within profiles concentrations were higher at 40 and 45 cm depth than at 15 and 20 cm.

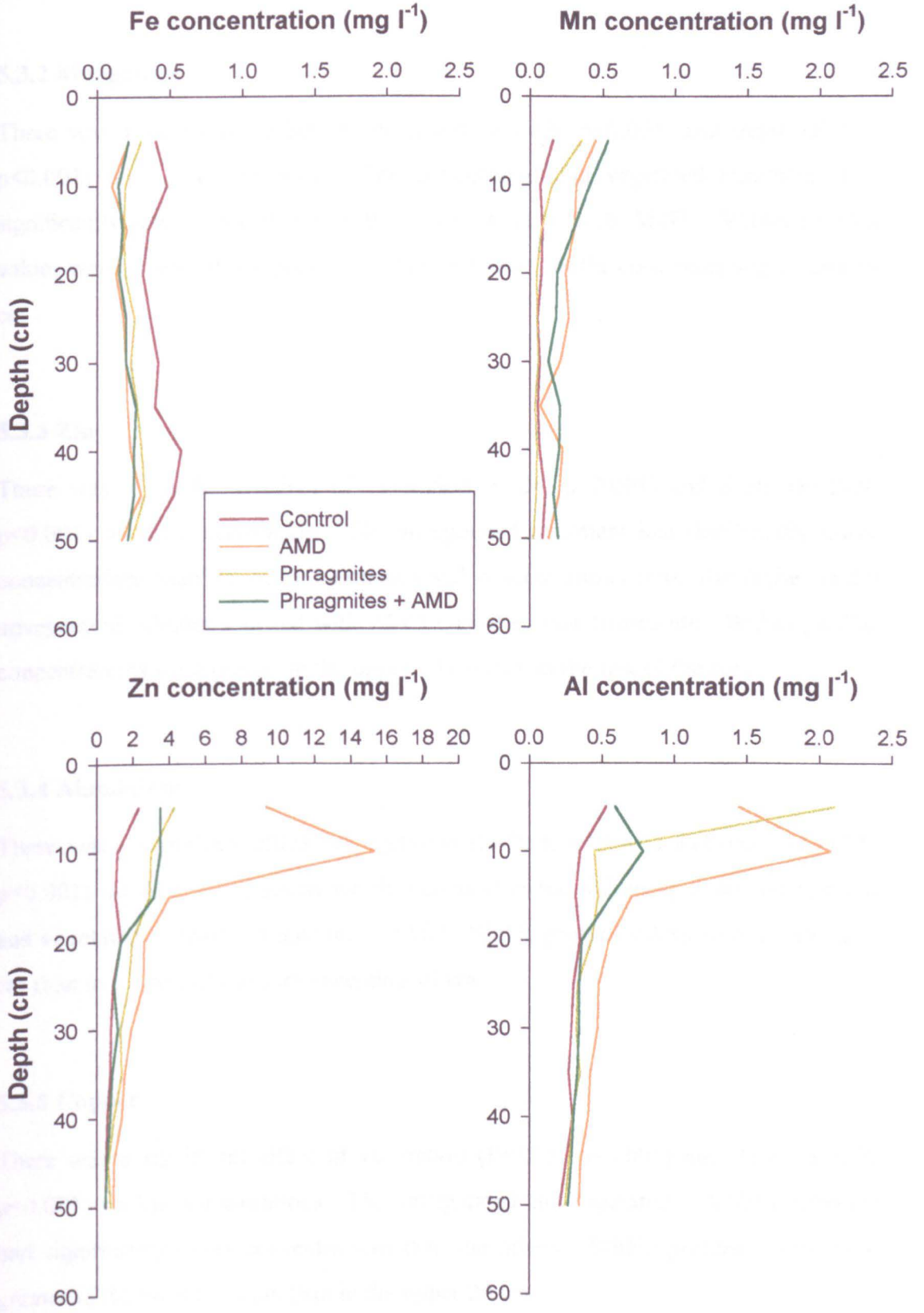


Figure 5.1. Fe, Mn, Zn and Al concentration profiles in wetland microcosms.

5.3.2 Manganese

There was a significant effect of vegetation ($F=20.8$, $p<0.001$) and depth ($F=4.5$, $p<0.001$) on Fe concentration. The unvegetated and vegetated treatments had significantly lower concentrations than those treated with AMD. Within profiles values were greater at a depth of 5 cm than in the rest of the core excepting 10 and 15 cm.

5.3.3 Zinc

There was a significant effect of vegetation ($F=25$, $p<0.001$) and depth ($F=18.9$, $p<0.001$) on Zn concentration. The unvegetated treatment had significantly lower concentrations than the other treatments and concentrations were also higher in the unvegetated cylinder supplied with AMD than the other treatments. Within profiles concentrations were greater in the upper 10 cm than in the rest of the core.

5.3.4 Aluminium

There was a significant effect of vegetation ($F=20.4$, $p<0.001$) and depth ($F=17.5$, $p<0.001$) on Al concentrations which increased in the following order unvegetated and vegetated + AMD < vegetated < AMD. Within profiles values were greater at 5 cm than in the rest of the core excepting 10 cm.

5.3.5 Copper

There was a significant effect of vegetation ($F=12.1$, $p<0.001$) and depth ($F=9.7$, $p<0.001$) on Cu concentrations. The unvegetated and vegetated + AMD treatments had significantly lower concentrations than the others. Within profiles values were greater in the bottom 10 cm than in the upper 20 cm.

5.3.6 Calcium

There was a significant effect of treatment ($F=226.6$, $p<0.001$), depth ($F=17.3$, $p<0.001$) and their interaction ($F=2.7$, $p<0.001$) on Ca concentrations. The

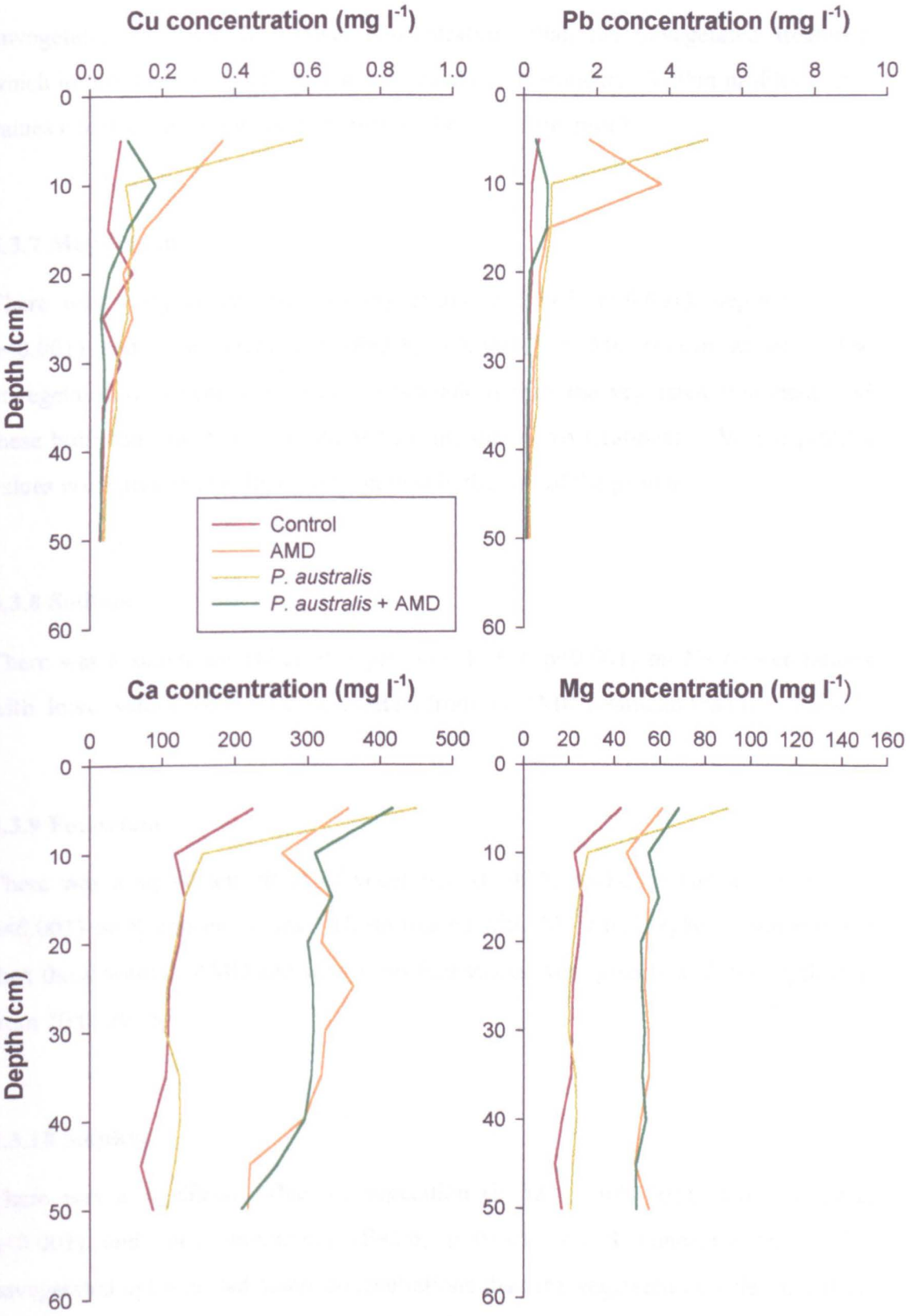


Figure 5.2. Cu, Pb, Ca and Mg concentration profiles in wetland microcosms.

unvegetated treatment had lower concentrations than the unvegetated treatment which in turn had lower values than the remaining treatments. Within profiles higher values occurred at a depth of 5 cm than in the rest of the profile.

5.3.7 Magnesium

There was a significant effect of vegetation ($F=289.3$, $p<0.001$), depth ($F=15.1$, $p<0.001$) and their interaction ($F=3.8$, $p<0.001$) on Mg concentrations. The unvegetated treatment had lower concentrations than the vegetated treatment, and these both had lower concentrations than the other two treatments. Within profiles values were greater at a depth of 5 cm than in the rest of the profile.

5.3.8 Sodium

There was a significant effect of vegetation ($F=8.9$, $p<0.001$) on Na concentrations with lower values recorded in porewaters from the AMD treatment than the others.

5.3.9 Potassium

There was a significant effect of vegetation ($F=40.8$, $p<0.001$) and depth ($F=3.5$, $p<0.001$) on K concentrations. Those treated with AMD had higher concentrations than those without AMD and within profiles values were greater at 5 cm depth than from 10 to 20 cm.

5.3.10 Sulphur

There was a significant effect of vegetation ($F=583.1$, $p<0.001$), depth ($F=12.1$, $p<0.001$) and their interaction ($F=2.6$, $p<0.001$) on S concentrations. The unvegetated cylinder had lower concentrations than the vegetated cylinder and these both had lower values of S than those treated with AMD.

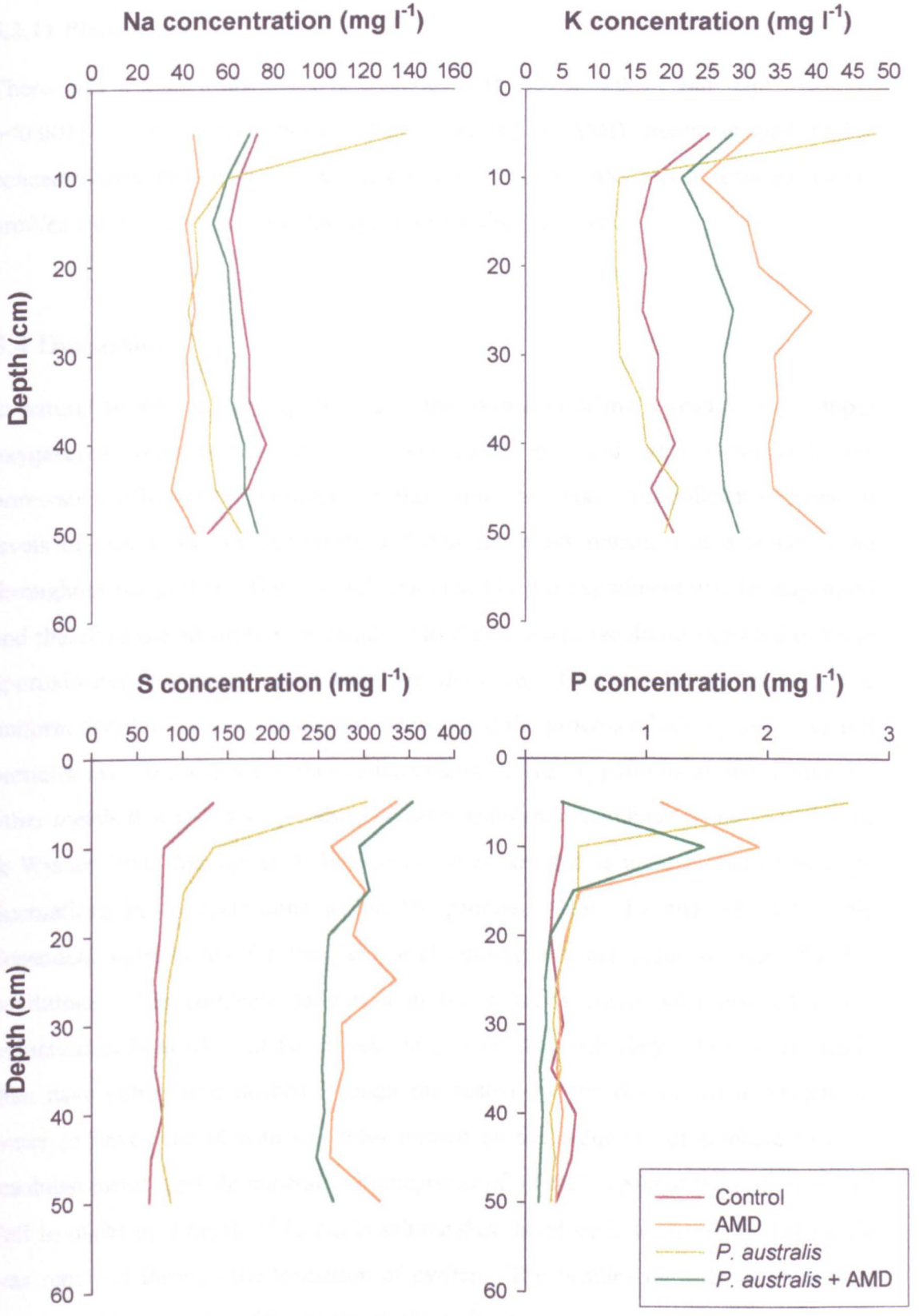


Figure 5.3. Na, K, S and P concentration profiles in wetland microcosms.

5.3.11 Phosphorus

There was a significant effect of vegetation ($F=12$, $p<0.001$) and depth ($F=8.7$, $p<0.001$) on P concentrations. The vegetated + AMD treatment had higher concentrations than either the vegetated or unvegetated + AMD treatments and within profiles values were greater in the upper 15 cm than at 50 cm.

5.4 Discussion

In natural systems it was suggested that the formation of metal oxides in the upper oxygenated layers reduced the concentration of iron and other elements in the porewaters (Chapter 4). However, in this study there was no significant decrease in levels of iron in the surface layers and concentrations remained at a similar level throughout the profile. The peat substrate used in this experiment was homogenised and therefore the adsorption of metals onto the sediment would be expected to be at approximately the same level throughout the core. This may be the cause of the uniform distribution of Fe through the cores, and the process of adsorption on to soil particles may be dominant in these microcosms. However, patterns of distribution for other metals that may also be adsorbed onto sediments including Cu and Zn (Henrot & Wieder 1990; Machemer & Wildeman 1992), are not as uniform and show large fluctuations in concentrations within the profiles. Both Fe and Mn are highly dependent upon redox for their chemical activity and are reduced under flooded conditions. The complete saturation of the columns could have resulted in the reduction of both Mn and Fe, thereby increasing their solubility. The metals could then have either been flushed through the system by the downward movement of water or have reacted with sulphides formed by the reduction of sulphate to form insoluble metal sulphide minerals. Boulegue *et al.* (1982) reported the conversion of FeS to pyrite by a depth of 12 cm in saltmarshes therefore it seems likely that the Fe was removed through the formation of pyrites. The profiles for sulphur show that concentration remains fairly constant throughout the core and so supporting the proposed explanation of metal sulphide formation. In the top 5 cm however, sulphur concentrations are much higher and this may be due to localised oxidising conditions which prevent the reduction of sulphate and therefore the formation of sulphide. In

this region the iron and manganese could also be oxidised thereby removing them from the porewaters which accounts for the low concentrations of these elements in the upper layer. The formation of Mn oxides may also increase the precipitation of Fe and cause the formation of x-ray amorphous and/or different crystalline iron oxide precipitates (Krishnamurti & Huang 1988).

The profiles for Mn, Al, Cu, Zn and Pb showed that concentrations were lower in the vegetated microcosm treated with AMD than unvegetated + AMD suggesting that plant growth may have had an effect on porewater concentrations. It has been suggested that the formation of iron oxides around the roots of wetland plants may also remove other metals from the surrounding environment through adsorption processes (Sundby *et al.* 1998). This is thought to be due to the high specific surface area of iron oxyhydroxides particularly those that are poorly crystalline (Stumm & Sulzberger 1992; Fortin *et al.* 1993). Amorphous plaque material has been reported to occur as iron plaques formed in field and laboratory conditions (Taylor *et al.* 1983; St Cyr *et al.* 1993). However, this process would be expected to be localised around the rooting layer and should not extend throughout the profile, although metals may adsorb onto the oxides as the water passes through the rooting zone as it percolates downwards. This would also help to explain the absence of a dip in concentration in the rooting zone as metals would not pass down into the lower layers. Concentrations of Al, Cu, Pb and Zn all decrease significantly below a depth of about 10 to 20 cm, the level of the rooting zone. However, this also occurs in those microcosms that are not planted with *P. australis* and therefore another process must be causing this pattern. Cu and Zn are known to form sulphides in reducing conditions (Shotyk 1988; Machemer & Wildeman 1992) and thus these elements may be removed below the oxygenated surface layer by reacting with sulphides. However, a similar pattern is also seen for Al and this does not form sulphide minerals.

In the microcosms treated with AMD there is a concentration of Al, Cu, P, Zn and Pb at a depth of 10 cm, which is evidently not caused by plant root activity as this pattern also occurs in unvegetated systems. If iron and manganese oxides form in the upper layers of the profile and other metals are adsorbed onto them, then in those systems treated with AMD there may not be enough adsorption sites for the level of metals in the system. Therefore there would be a higher concentration of these elements in the

upper levels of systems treated with AMD. At lower layers these elements would then be removed by the formation of sulphide minerals. Al however does not form sulphide minerals and therefore must be removed by another process. Trace metals have also been shown to adsorb onto the surface of pyrite and therefore could be responsible for the removal of Al and other metals from the porewaters (Kornicker & Morse 1991).

In the upper layers, oxidising conditions causes the sulphur to occur as sulphate which at low pH and high Al concentrations may form $AlSO_4$ minerals therefore explaining the low concentrations of Al in the upper layers of those microcosms treated with AMD. At lower levels, at about 10 cm depth reducing conditions dominate and this would allow the reduction of sulphate to sulphide thereby releasing Al into the system, a peak of Al occurs in this layer in those treated with AMD. A corresponding increase in sulphur is not evident as this would react with reduced Fe and Mn to form sulphide minerals thereby remaining at a low level. However, if the formation of aluminium sulphate was the cause of the pattern seen for Al it would be expected to occur in all treatments, which is not the case. In addition aluminium sulphate is highly soluble and therefore formation of this compound would not remove Al from the interstitial waters.

The concentrations of Ca in the cores shows that levels are higher in those microcosms supplied with AMD. The amount of Ca added to all the systems was the same and therefore it must have been due to the AMD specifically. Due to the type of compounds used to produce the artificial AMD the concentration of sulphate ions added is higher in the systems treated with AMD and this is evident in the profiles for sulphur content. This could have reacted with Ca to form $CaSO_4$ which is highly soluble in water therefore increasing the solubility of Ca in the system. The levels remain at a similar level throughout the profile however, suggesting that oxidising conditions and the presence of sulphate occurs down the entire core. If this is the case the formation of Fe and Mn oxides in the core could explain the low concentrations of both these elements throughout the core. However, upon dissection there was no evidence of the characteristic orange colour of Fe oxides throughout the core and therefore this is unlikely.

5.5 Conclusions

An attempt was made to investigate the distribution of metals within wetland systems, and specifically the role of root activity using laboratory based microcosms. The use of such systems aimed to remove additional factors that control the activity of metals, including sediment type, input and output of metals and the direction of flow. Although patterns of metal distribution obtained were clearer than those from the field (chapter 4), the proposed explanations for the profile distributions of metals do not apply to all metals in all situations. However, it is suggested that there are two distinct layers within these systems, the upper oxidising layer (approximately 10 cm deep) and the lower reducing layer. In the upper region oxidation of Fe and Mn remove these elements from the porewaters, and the occurrence of sulphur and sulphate in this region gives high concentrations of S in the interstitial waters. In the lower reducing conditions, Fe and Mn oxides are reduced and this releases Fe and Mn into porewaters, but these react with reduced sulphur (sulphide) to form metal sulphides and pyrite. The concurrent formation of Zn, Cu and Pb sulphides also remove these elements from solution and the adsorption of trace metals including Al also achieve this result. However, the lower concentrations in some elements in the vegetated microcosms in comparison with the unvegetated treatments suggest that plant growth may also affect metal concentrations in wetland systems, but the dominance of chemical processes makes it difficult to identify these. It is likely that different processes have varying significance which is dependent upon the metal species investigated and their potential interactions. Thus, porewater chemistry is not a suitable method by which to determine the activity and role of root activity within wetland systems.

6. The effect of iron plaque and pH on uptake of Cu and Mn in *P. australis*.

6.1 Introduction

The formation of iron plaques around the roots of wetland plants may constitute an adaptation to stressed environments. In flooded areas the reduction of iron and manganese compounds results in the accumulation of high concentrations of these bioavailable elements. In addition, particularly in acid soils, there may be potentially phytotoxic quantities of metals or metalloids. Iron oxides present in soils and sediments have high specific surface areas and possess -OH functional groups which are capable of reacting with metals and other cations and anions (Kuo 1986). It is possible that iron hydroxides forming on the roots of wetland plants have similar properties and may therefore immobilise and prevent the uptake of phytotoxic metals.

A detailed review of the literature investigating the uptake of metals in a variety of wetland species is given in Chapter 1. It was demonstrated that no definitive conclusions regarding the role of plaque could be gained from previous work due to inconsistencies in research. In particular the use of a wide range of plants species, cultural techniques and chemical analysis of plant material has given rise to conflicting results and information.

It was suggested in Chapter 3 that the formation of iron plaque on roots may vary both with plant species and time of year, and as a result the availability of metals potentially associated with the iron oxides also changes. However, the techniques used in the previous chapters have not allowed a detailed investigation of the chemical processes occurring within the rhizosphere particularly in relation to iron plaque formation.

The effect of pH on plaque formation has not been extensively studied, but generally it has been reported that iron plaque is positively correlated with pH. However, the majority of these studies were undertaken in an extremely narrow pH range between 5 and 7 (Macfie & Crowder 1987; St Cyr & Crowder 1989) which is also the pH at which investigations of plaque chemistry, mineralogy and metal uptake are usually

carried out (Bacha & Hossner 1977; Chen *et al.* 1980 a,b; Otte *et al.* 1987; Johnson-Green & Crowder 1991; Greipsson & Crowder 1992; Greipsson 1994; Ye *et al.* 1997a,b, 1998a). In the few isolated studies that have used a wider range of pH (3 to 8) the results have reported the positive correlation between pH and plaque formation (Taylor *et al.* 1983; Crowder & Coltman 1993). However, St Cyr & Campbell (1996) found that there was a weak negative correlation between sediment pH and root plaque over the pH range 5.8 to 7.5 which they suggested was due to the reduced availability of iron at higher pH.

It is evident therefore that there is a wide range of conflicting information in the literature regarding the chemistry and mineralogy of plaques, their role in the prevention of metal uptake in species, their ability to immobilise metals and the importance of the chemical environment on plaque formation. Wetland plants have been used extensively in constructed wetlands for the removal of metals from wastewaters. The acidity of these environments may affect the extent and type of plaque formed on the roots of plant and this in turn could affect the uptake of potentially phytotoxic metals into plant tissues. These variables could influence the growth of wetland plants in such environments and thus their ability to remove metals from contaminated waters. However it has been shown to be impossible to determine this through the chemical analysis of porewaters surrounding the roots of plants growing in natural and laboratory conditions (Chapter 4 and 5) due to the complexity of soil systems. A more direct approach using solution culture studies was needed to determine the specific effects of plaque on metal uptake.

The aims of this chapter therefore were to

- characterise the chemistry and mineralogy of plaques formed in the field and laboratory
- determine the effect of plaque on the uptake of heavy metals into plant tissues
- determine the effect of pH on plaque development and metal uptake

6.2 Materials and Methods

6.2.1 The chemical composition and structure of iron plaques

Field specimens

In order to compare the characteristics of plaques formed in the field with those formed in the laboratory it was necessary to select a number of metal-contaminated and uncontaminated field sites.

The contaminated sites needed to have received metal-rich contaminated mine drainage for a number of years, and to contain a living, established community of wetland plants. At Parys Mountain (described in chapter 3) the species collected was *Phragmites australis* from a stand growing in the main outlet stream from the mine site. Woolley Colliery is a derelict coal mine which discharges metal-rich drainage waters into a constructed wetland. This wetland is well-established and contains *P.australis* and *Typha latifolia*, samples of which were collected in September 1998.

It was also necessary to collect material from an uncontaminated site and samples of *P. australis* were collected from Upton Fen, Norfolk. This is a revegetating turf pond fed by uncontaminated water containing low concentrations of N and P.

Laboratory specimens

The characteristics of iron plaques formed in the laboratory were studied as part of a larger experiment designed to investigate the effects of iron plaques on the uptake of metals by *P. australis.*

Ten uniformly-sized seedlings of *P.australis* were selected and transplanted into a blackened perspex vessel (28 x 17 x 9 cm) containing 1.5 L of 10% Rorison's nutrient solution. This was repeated for 36 units allowing for three replicates of all treatments, arranged in a randomised block design. The seedlings were grown for 63d, the Rorison's solution being changed every 3d. At the end of this period the pH of the solution in 18 of the containers was reduced gradually to 3.5 using 0.1 M H_2SO_4, over a period of 7d.

Each set of units at pH 3.5 and 6 was split into two subsets consisting of nine containers. In one of these subsets plaque formation was induced and in the remaining

sets the roots were left unplaqued. Plaque formation was induced on the roots of seedlings prior to metal treatment through the addition of 50 mg/L of Fe supplied as ferrous ammonium sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$) in nutrient solution which did not contain potassium phosphate thereby preventing the interaction of Fe with P. In addition, the seedlings were placed in distilled water of the appropriate pH for 12 hrs before plaquing to prevent a similar interference of P and were left for 7d. Plaque was visible on roots as an orange-brown deposit after 7d. All seedlings were grown for a further 5d in 10% Rorison's solution prior to metal treatment. The two metals added were Cu ($CuSO_4.\ 5H_2O$) and Mn ($MnSO_4.\ 4H_2O$), both at a concentration of 0.5 mg/L. These were added to the appropriate containers to give the following conditions: (1) no plaque, Cu, pH 3.5, (2) no plaque, Cu, pH 6.0, (3) plaque, Cu, pH 3.5, (4) plaque, Cu, pH 6.0, (5) no plaque, Mn, pH 3.5, (6) no plaque, Mn, pH 6.0, (7) plaque, Mn, pH 3.5 and (8) plaque, Mn, pH 6.0. Control treatments contained no additional metals. During metal exposure solutions were changed every 3d. Roots were re-plaqued at 21d and 50d to ensure coverage of roots with plaque was maintained. Prior to the repeated exposure of roots to iron, the seedlings were placed in distilled water for 6h before exposure to 50mg/L Fe for 3d.

At the end of the experiment all plants were harvested and divided into roots and shoots. A representative root sample from each of the treatments was isolated and prepared for SEM analysis (2.1.4).

6.2.2 Metal uptake in field specimens

In order the determine whether the presence of iron plaque can prevent the uptake of potentially phytotoxic metals into plant tissues it was necessary to collect specimens of plants growing in contaminated conditions. Specimens collected for the SEM analysis (6.2.1) were used for this purpose. After collection the plants were split into roots and shoots, the shoots were prepared for acid digestion (2.1.3) and roots not used for the SEM were also prepared for acid digestion. This allowed direct comparison of the data from the SEM and chemical analysis.

6.2.3 The effect of iron plaque and pH on the uptake of Cu and Mn

The hypothesis that the presence of iron plaque on roots can reduce the uptake of metals in wetland plants was tested using hydroponic culture which provides simple plant-solution systems without any accessory effects of soils or sediments (6.2.1).

Data on metal concentrations were analysed using a combination of t-tests and 2-way ANOVA followed by a multiple comparison Tukey test. Where necessary, data transformations were carried out to reduce heteroscedasticity, usually either $\log_e$ or $\log_{10}$ values.

6.3 Results

6.3.1 Morphology of iron plaques

Field Specimens

Roots of *P. australis* collected from Woolley Colliery possessed an orange-brown coating on the surface which will be referred to as the iron plaque. Under the SEM it was evident that this coating took the form of an amorphous particulate deposit which was present both on the epidermal cells and attached to the root hairs (Plate 6.1). The plaque was not ubiquitous and areas of the root were evident which were 'clean'. In cross section the plaque did not penetrate into the epidermal cells, and cell casts and polyhedra as reported by Chen *et al.* (1980b) were not present.

Roots of *T. latifolia* from the same site also possessed an orange coating which proved to have similar physical characteristics to those on *P. australis* (Plate 6.2). The plaque differed however, in that it was absent in only very isolated areas of the root. In addition there was evidence of a plaque-type material in a small number of the epidermal cells. Where this occurred the precipitate appeared to have grown inwards into the cell cavity from the inner cell wall.

Phragmites australis collected from Parys mountain possessed a clear orange coloration of the root similar to those of Woolley Colliery. Under the SEM the

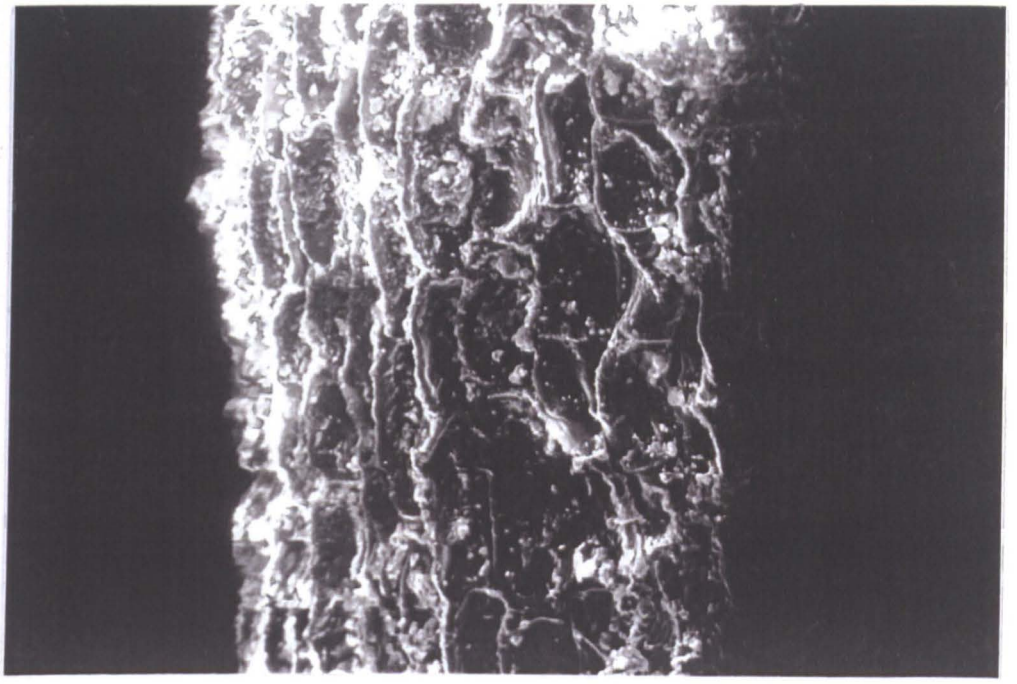


Plate 6.1. Plaque deposits on *P. australis* roots from Woolley Colliery (x 600).

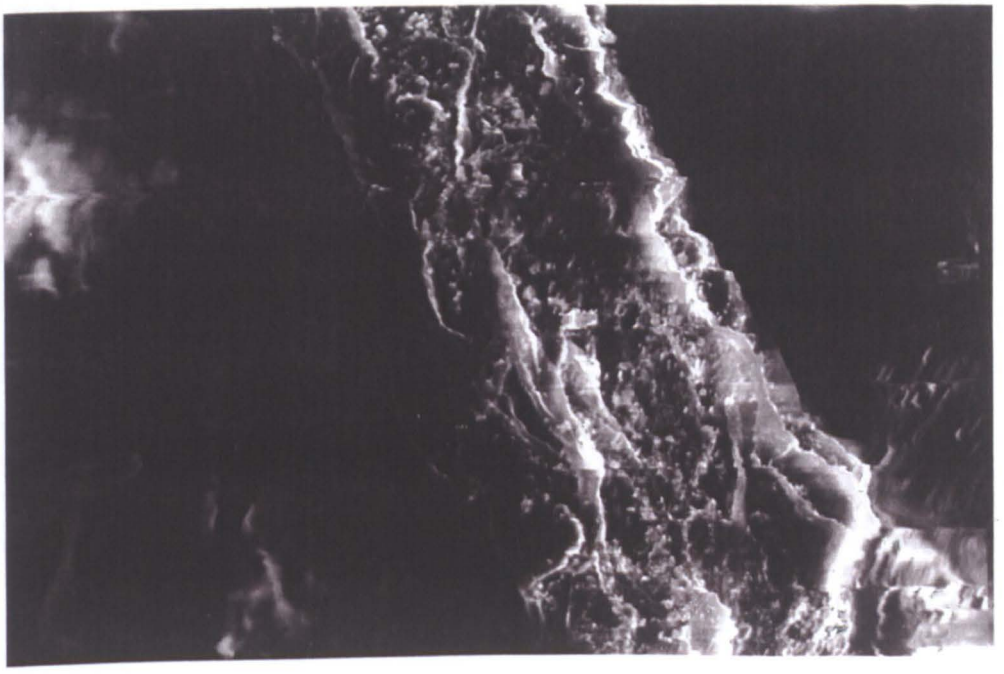


Plate 6.2. Plaque deposits on *T. latifolia* roots from Woolley Colliery (x 500).

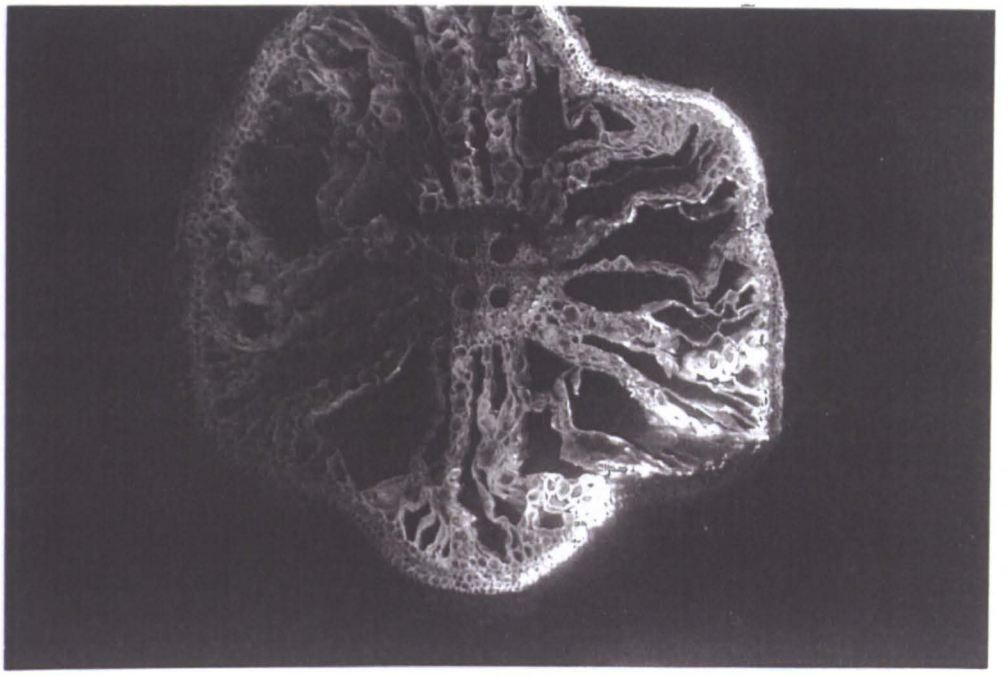


Plate 6.3. Cross section of a *P. australis* root from Woolley Colliery (x 55).

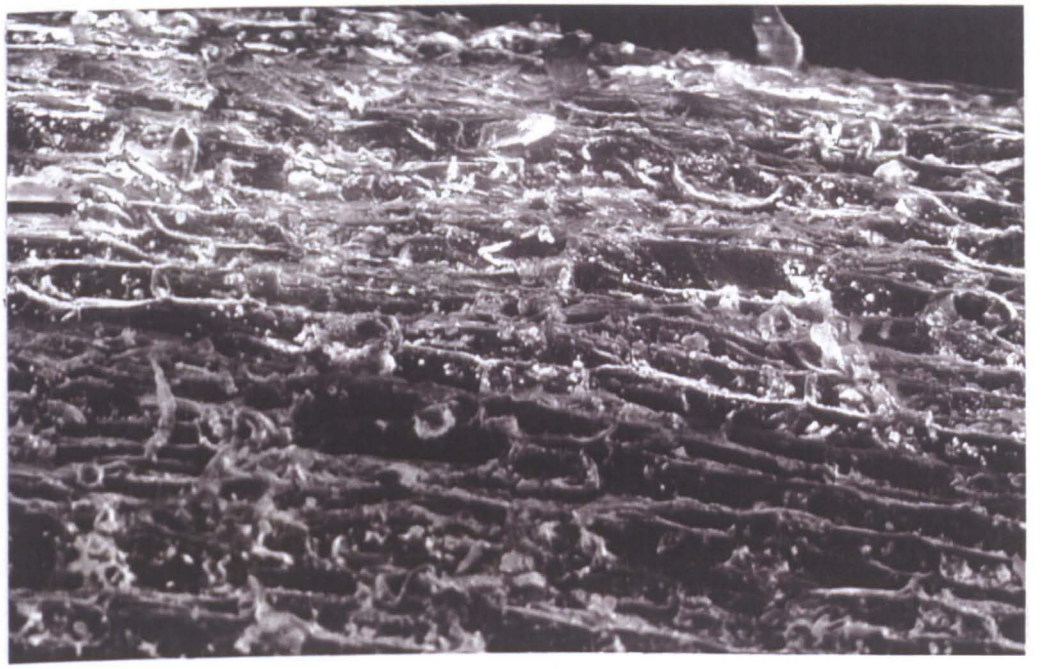


Plate 6.4. Plaque deposits on *P. australis* roots from Parys Mountain (x 300).

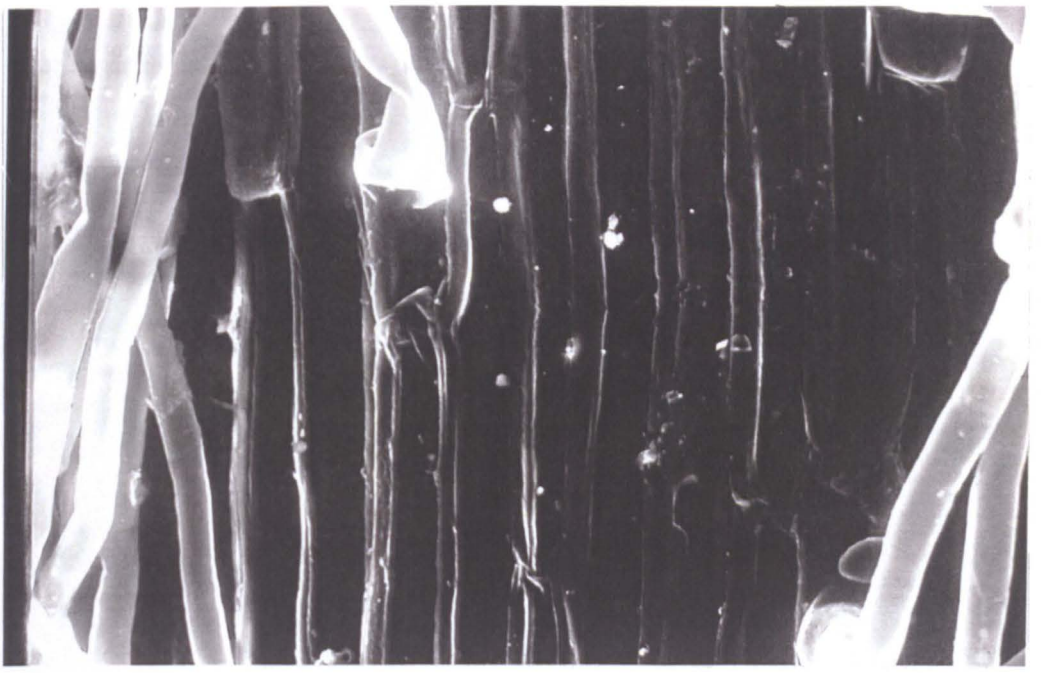


Plate 6.5. Unplaqued roots of *P. australis* from Norfolk (x 650).

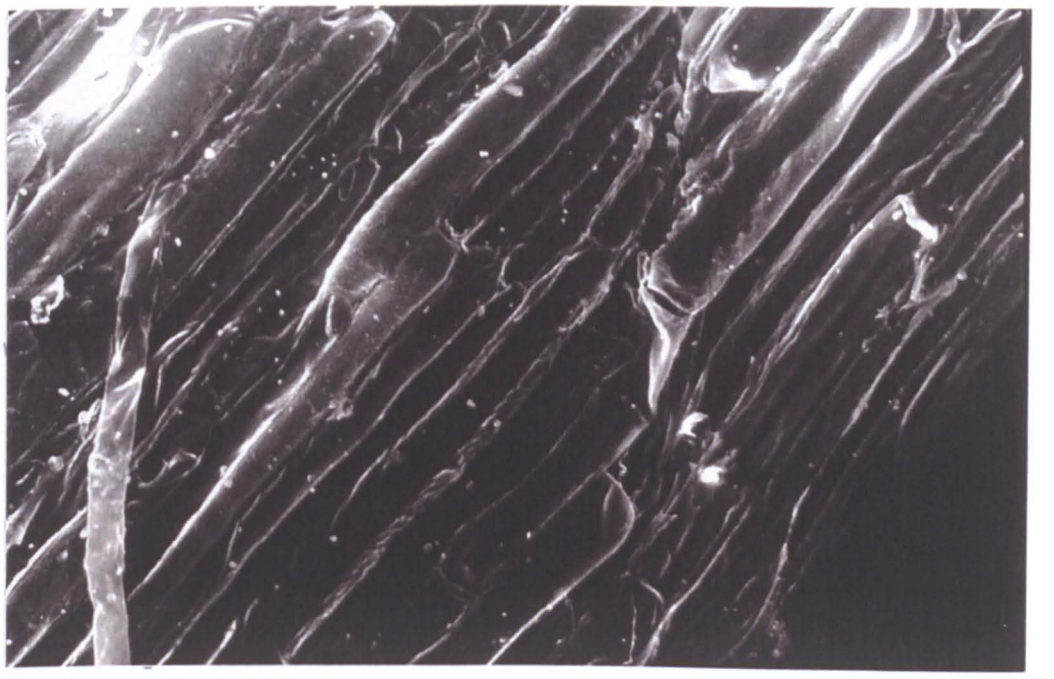


Plate 6.6. Unplaqued roots of *P. australis* from Norfolk (x 400).

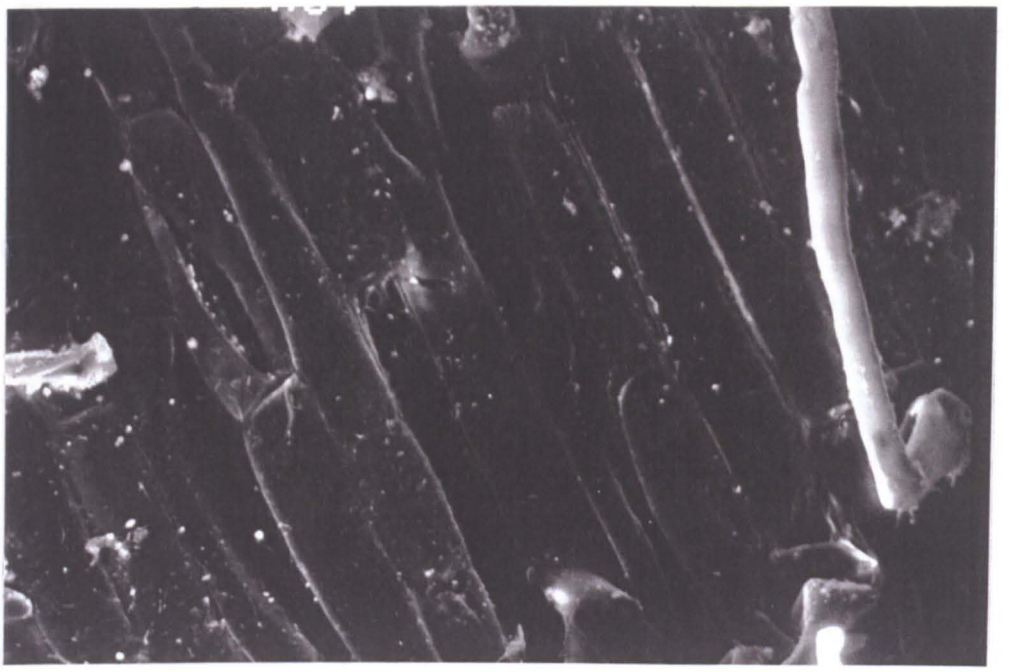


Plate 6.7. Unplaqued roots of *P. australis* grown in the laboratory at pH 6.0 (x 500).

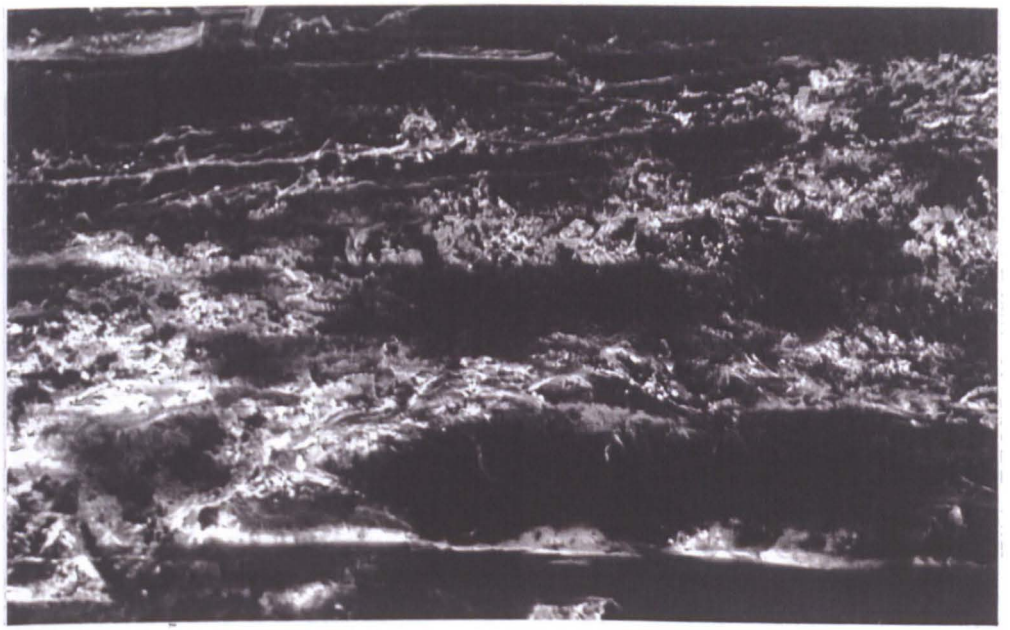


Plate 6.8. Plaque deposits on roots of *P. australis* grown in the laboratory at pH 6.0, supplied with 50 mg l^{-1} Fe (x 500).

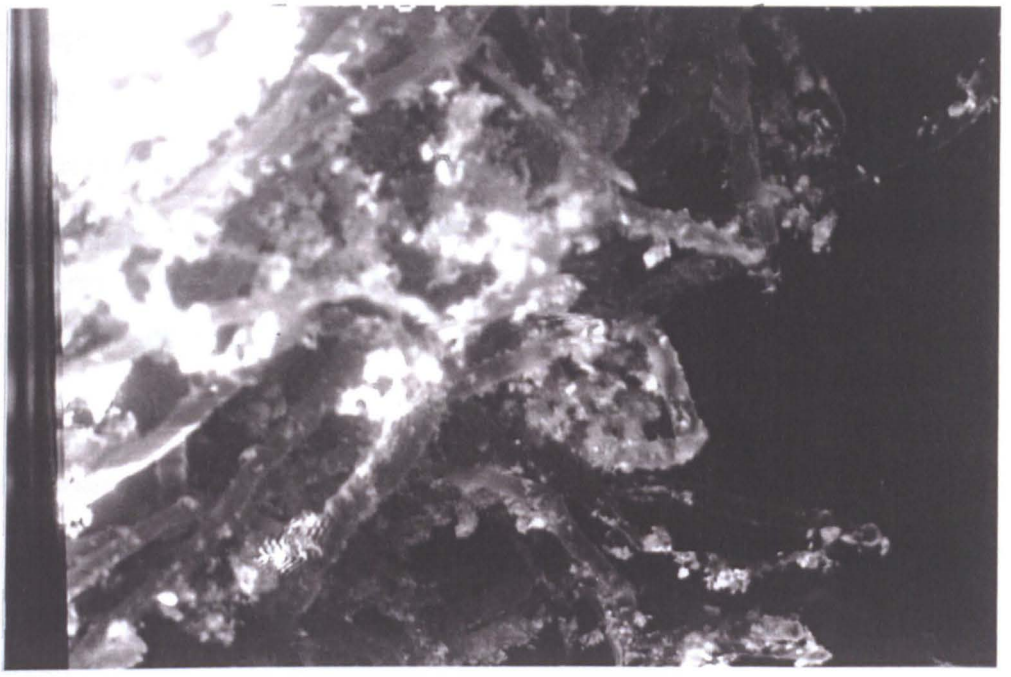


Plate 6.9. Plaque deposits on roots of *P. australis* grown in the laboratory at pH 6.0, supplied with 50 mg l^{-1} Fe (x 400).

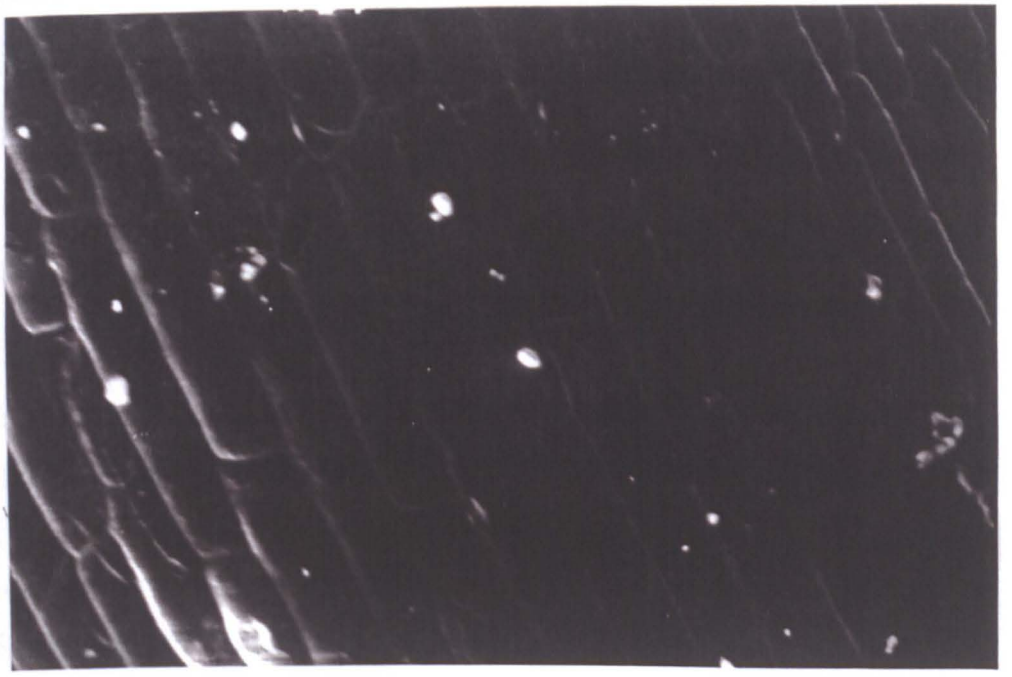


Plate 6.10. Roots of *P. australis* grown in the laboratory at pH 6.0, supplied with 50 mg l^{-1} Fe (x 650).

roots showed normal wetland root characteristics with good aerenchyma development (Plate 6.3). The orange deposit or plaque was present as an amorphous covering of the epidermis of the root (Plate 6.4). This was extensive and there were few areas where the plaque was absent. No evidence of polyhedra or cell casts were found.

The roots derived from the uncontaminated site at Norfolk were white in colour and under the SEM there was no evidence of any granular material on the root surface in any area. The epidermal cells were clearly visible and showed no signs of damage or cell decay (Plates 6.5 & 6.6).

Laboratory specimens

In the absence of plaque, the roots were white in colour and under the SEM there was no evidence of a deposit on the surface (Plate 6.7). Small particles were evident in isolation but these were probably clay particles.

When iron plaque was formed the roots had an orange-brown colour and under the SEM this appeared as a particulate covering of the epidermal cells (Plate 6.8). Plaque material was also evident around the root hairs (Plate 6.9). The coating was not uniform and large areas of the root were free of plaque deposits (Plate 6.10). In cross section there was no evidence of penetration of the plaque into the epidermal cells.

In those roots supplied with Mn only, small particles were found that resembled iron plaque, these however were extremely rare.

6.3.2 Chemical composition of iron plaques

Field specimens

EDS analysis of the 'clean' areas of the roots of *P. australis* from Woolley Colliery gave a chemical signature that was dominated by chloride, with minor amounts of Si, Ca, S and Na. There was also a trace signal of Fe and Mn (Figure 6.1). This contrasted with the chemistry of the plaque material which was composed mainly of Si together with significant amounts of Fe and Al (Figure 6.2). Other areas of plaque gave a slightly different signature with Mn dominating together with lower levels of

Ca and Fe (Figure 6.3). In isolated areas copper was also found in the plaque deposits.

The chemical signatures produced from the root sections of *T. latifolia* were similar, with the plaque composed mainly of Si, Fe and Al. Areas of plaque were also found that had higher levels of Ca with Mn and Fe.

EDS analysis of the roots of *Phragmites australis* from Parys mountain gave similar results to those of Woolley Colliery. The plaques were predominantly composed of Fe and S (Figure 6.4), but in some areas were formed of Fe, Al and S (Figure 6.5). There was no evidence for any occurrence of Cu, Mn or Zn with the plaque material.

EDS analysis of the roots from Norfolk showed that the roots were predominantly composed of Si and there was no evidence of Fe precipitation on the root surface (Figure 6.6)

Laboratory specimens

In roots supplied without any additional metals, X-ray analysis revealed the presence of K, S and Ca only. When roots were supplied with iron only, the chemical signature was very different with a strong signal for Fe, together with P and S (Figure 6.7). The same chemistry was recorded for the precipitate formed when iron was supplied in combination with copper or manganese. No signals for either of these metals were evident (Figures 6.8 & 6.9). Phosphorus and sulphur varied in their relative abundance but were always present.

When manganese or copper were supplied without iron there was no signal recorded for either metal on the root surface. However, a very small number of particles were found on the surface of those roots supplied with Mn alone and analysis revealed that they were composed of Si, K, Mn and Fe. No differences were noted between those plaques formed in the two pH conditions.

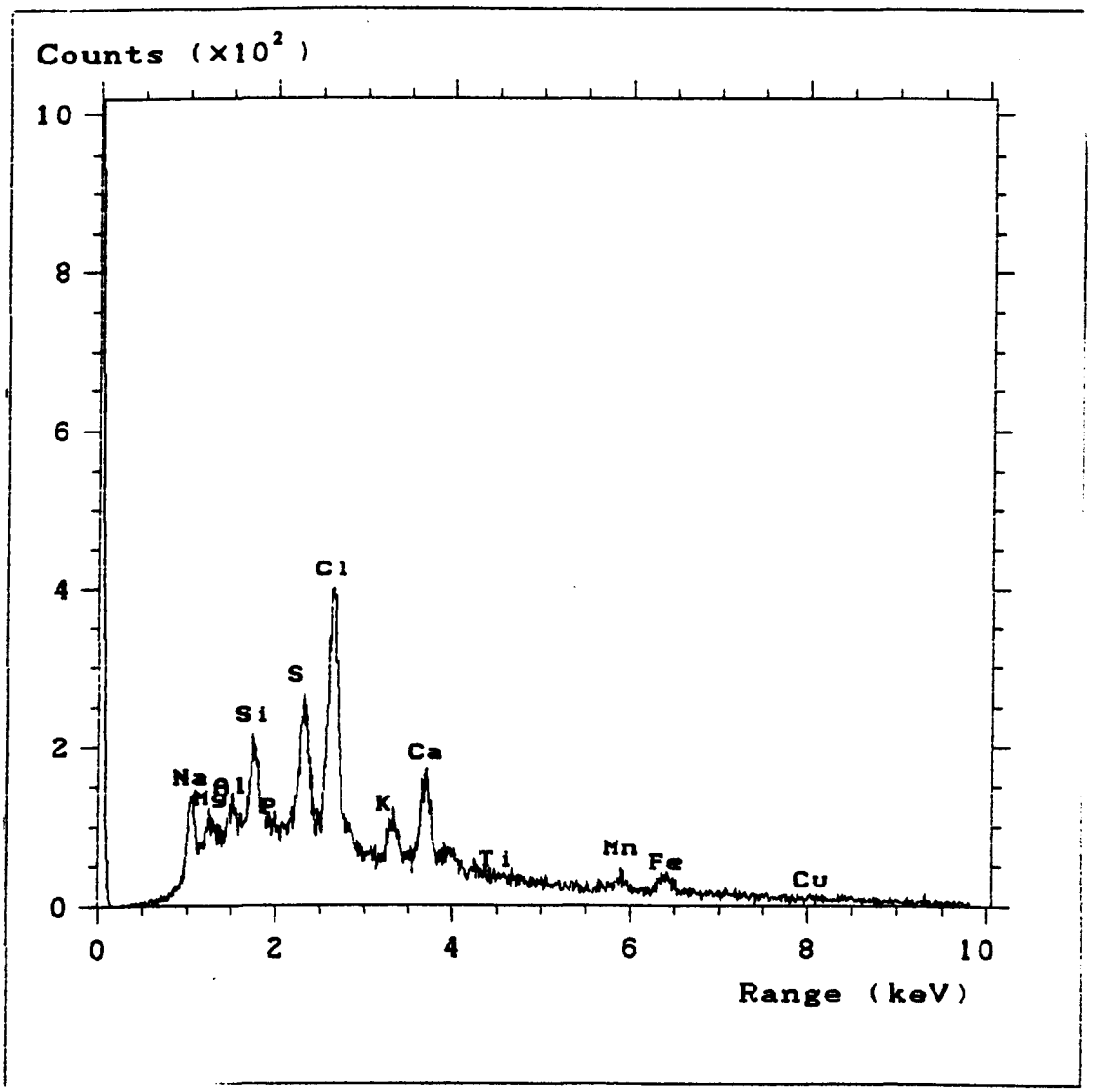


Figure 6.1. Composition of an unplaqued area on roots of *P. australis* from Woolley Colliery.

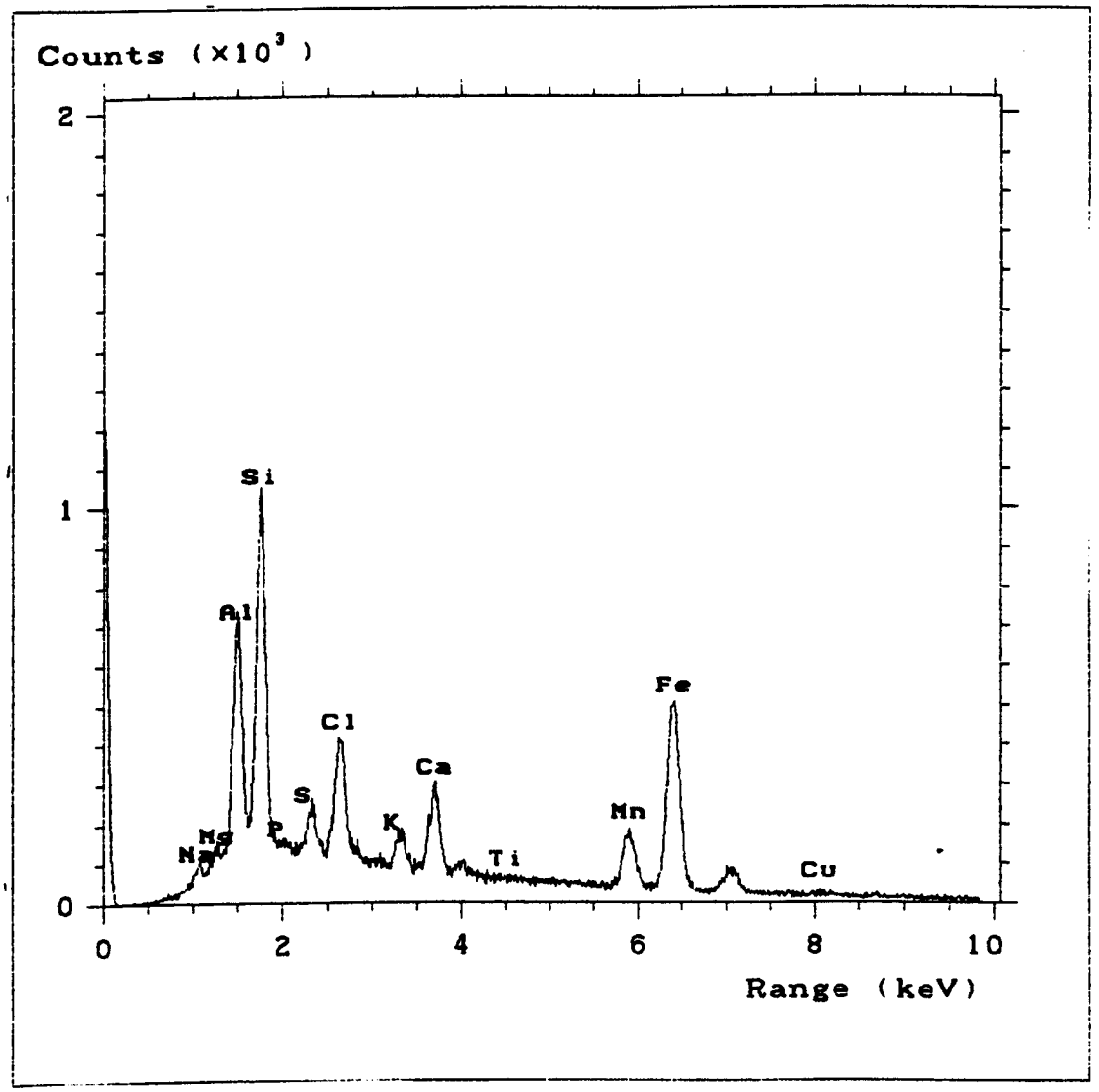


Figure 6.2. Composition of plaque deposits on roots of *P. australis* from Woolley Colliery.

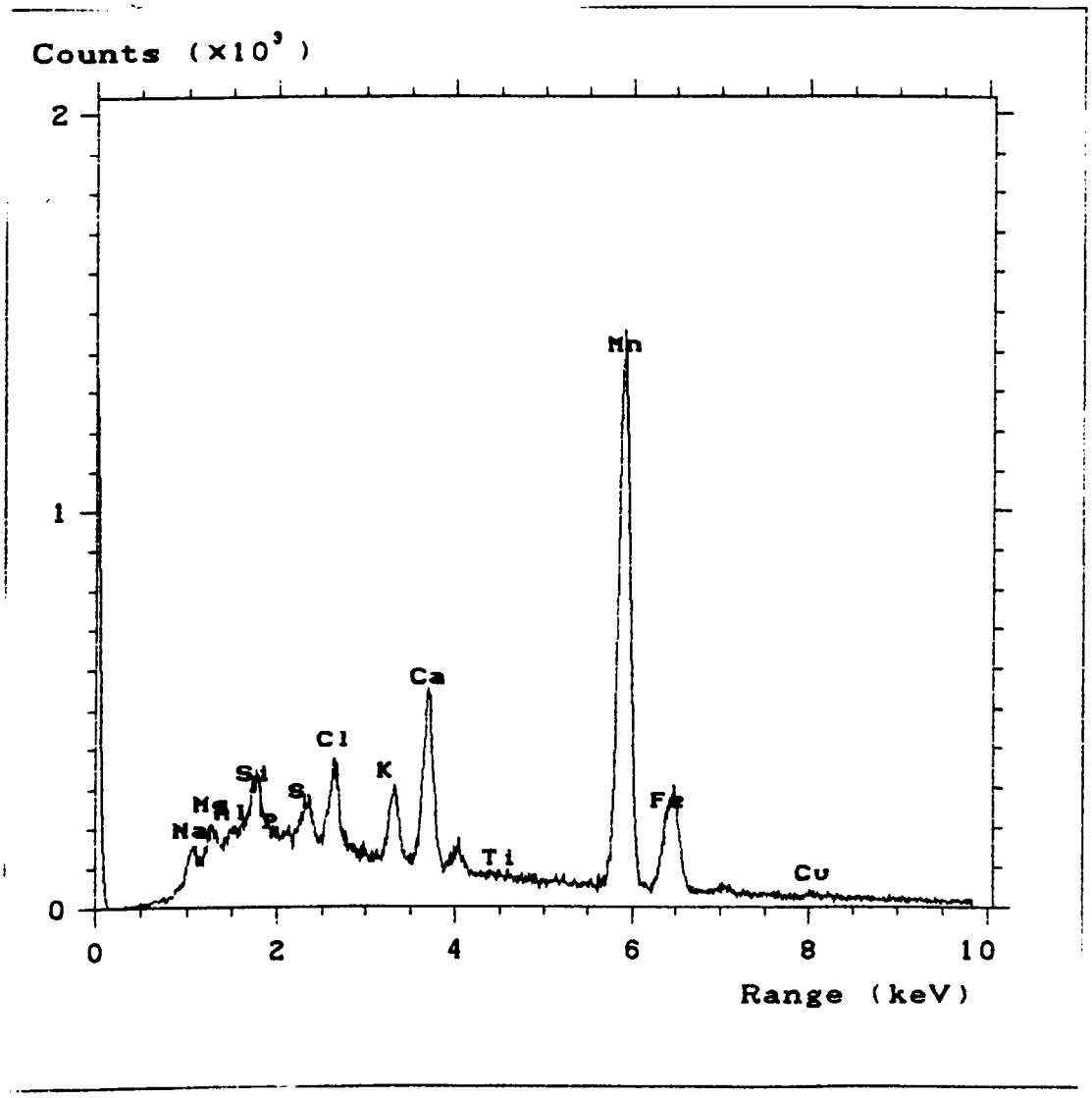


Figure 6.3. Composition of plaque deposits on roots of *P. australis* from Woolley Colliery.

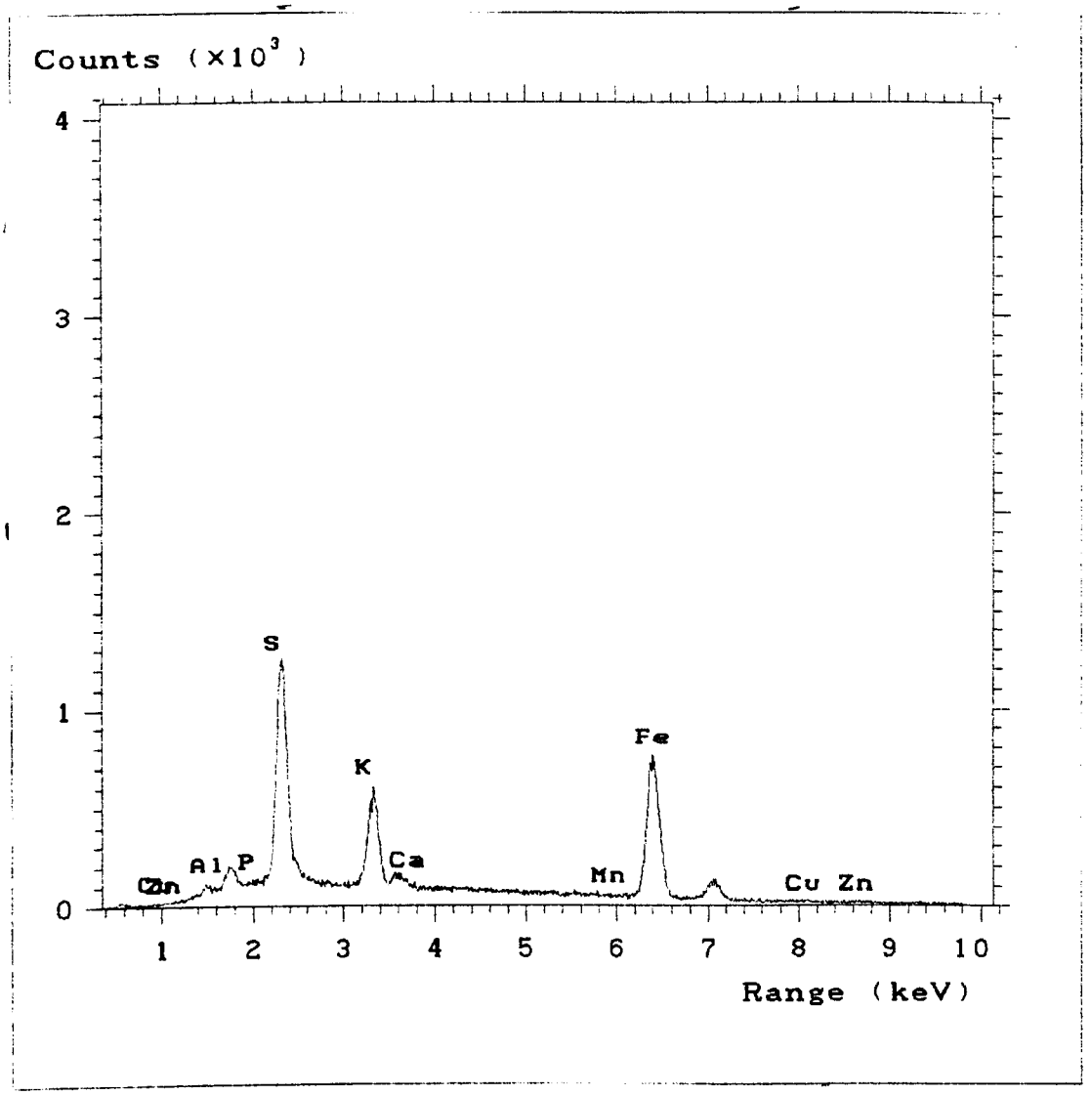


Figure 6.4. Composition of plaque deposits on roots of *P. australis* from Parys Mountain.

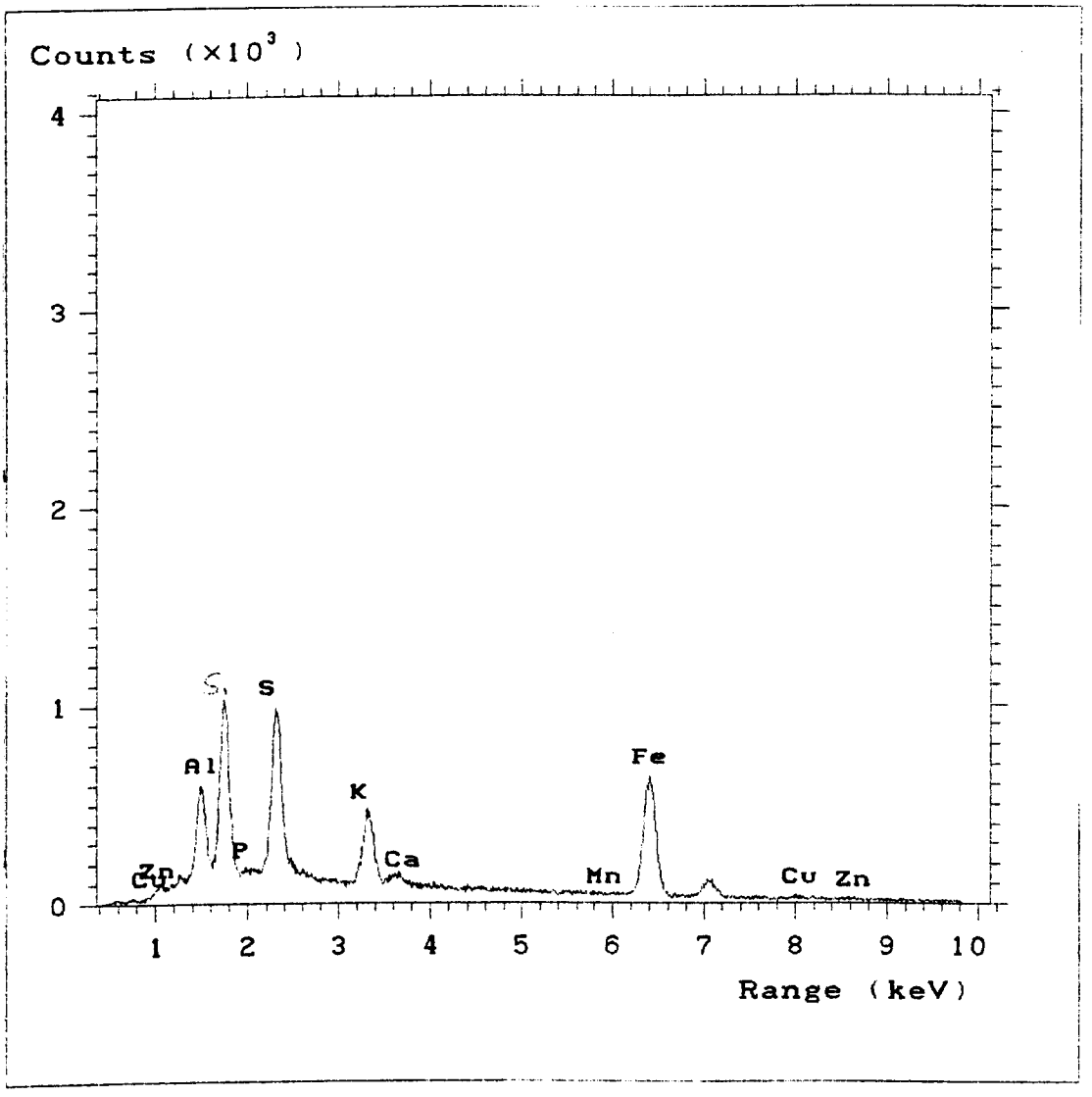


Figure 6.5. Composition of plaque deposits on roots of *P. australis* from Parys Mountain.

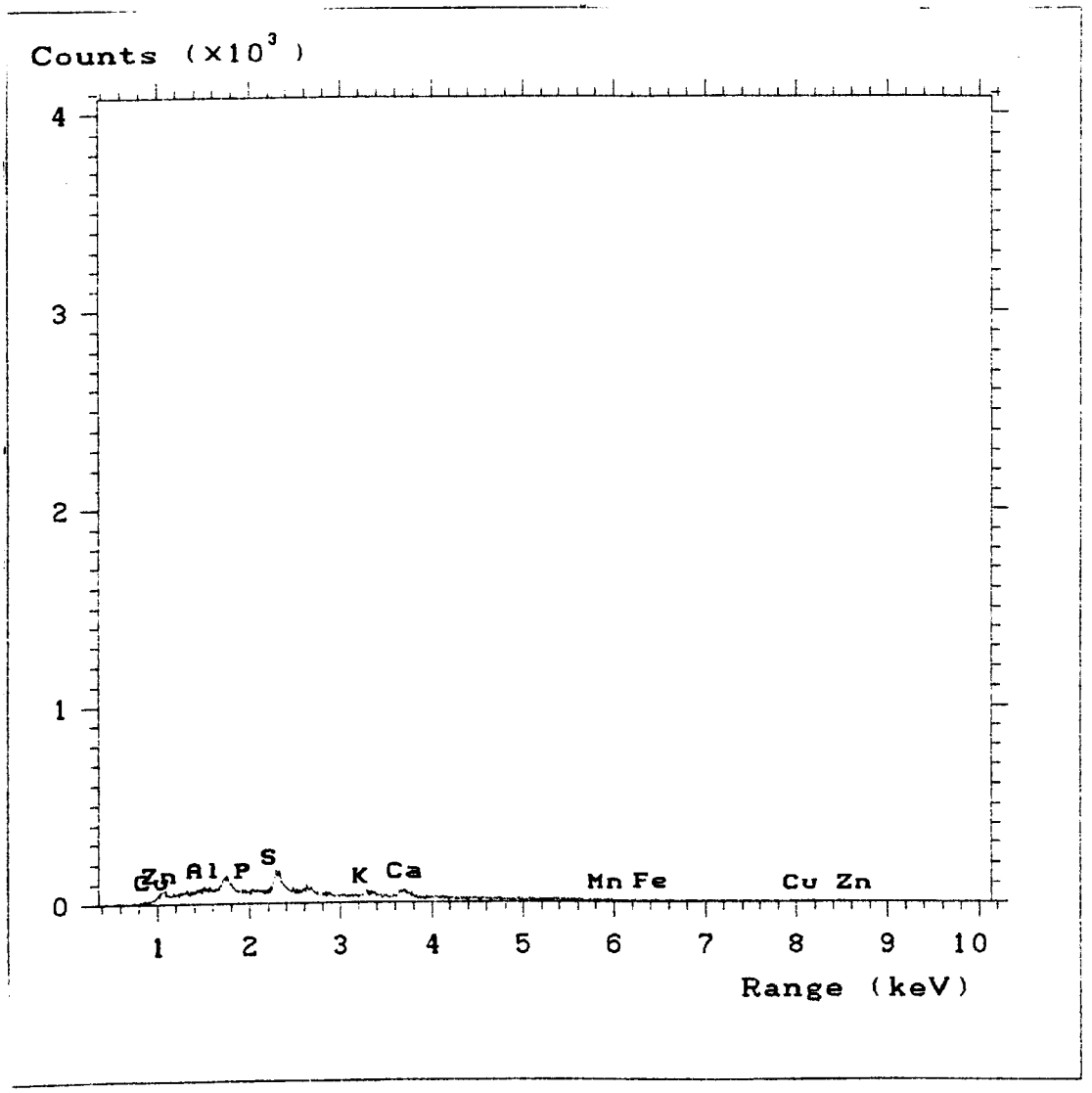


Figure 6.6. Composition of unplaqued roots of *P. australis* from Norfolk.

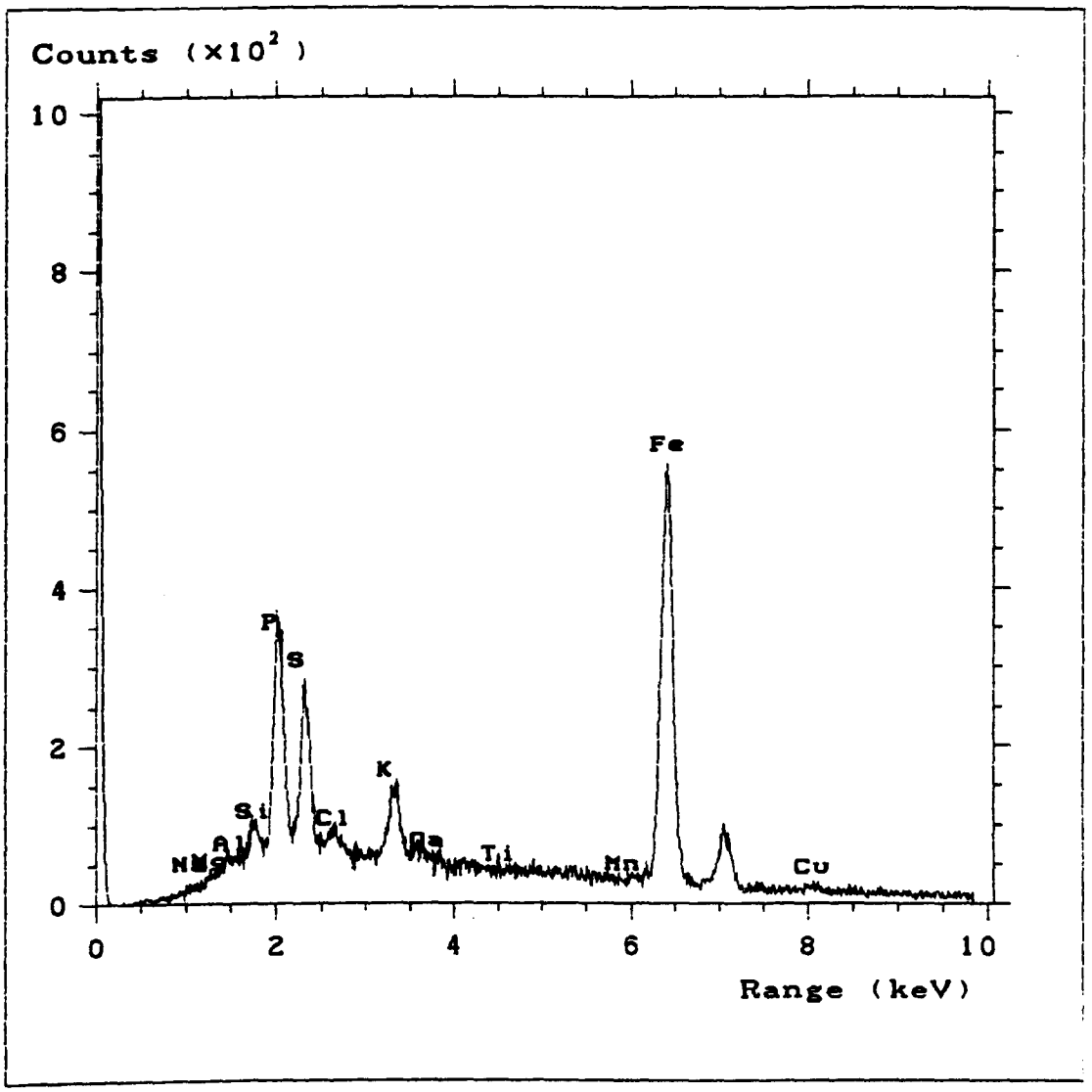


Figure 6.7. Composition of plaque deposits on roots of *P. australis* grown in the laboratory at pH 3.5.

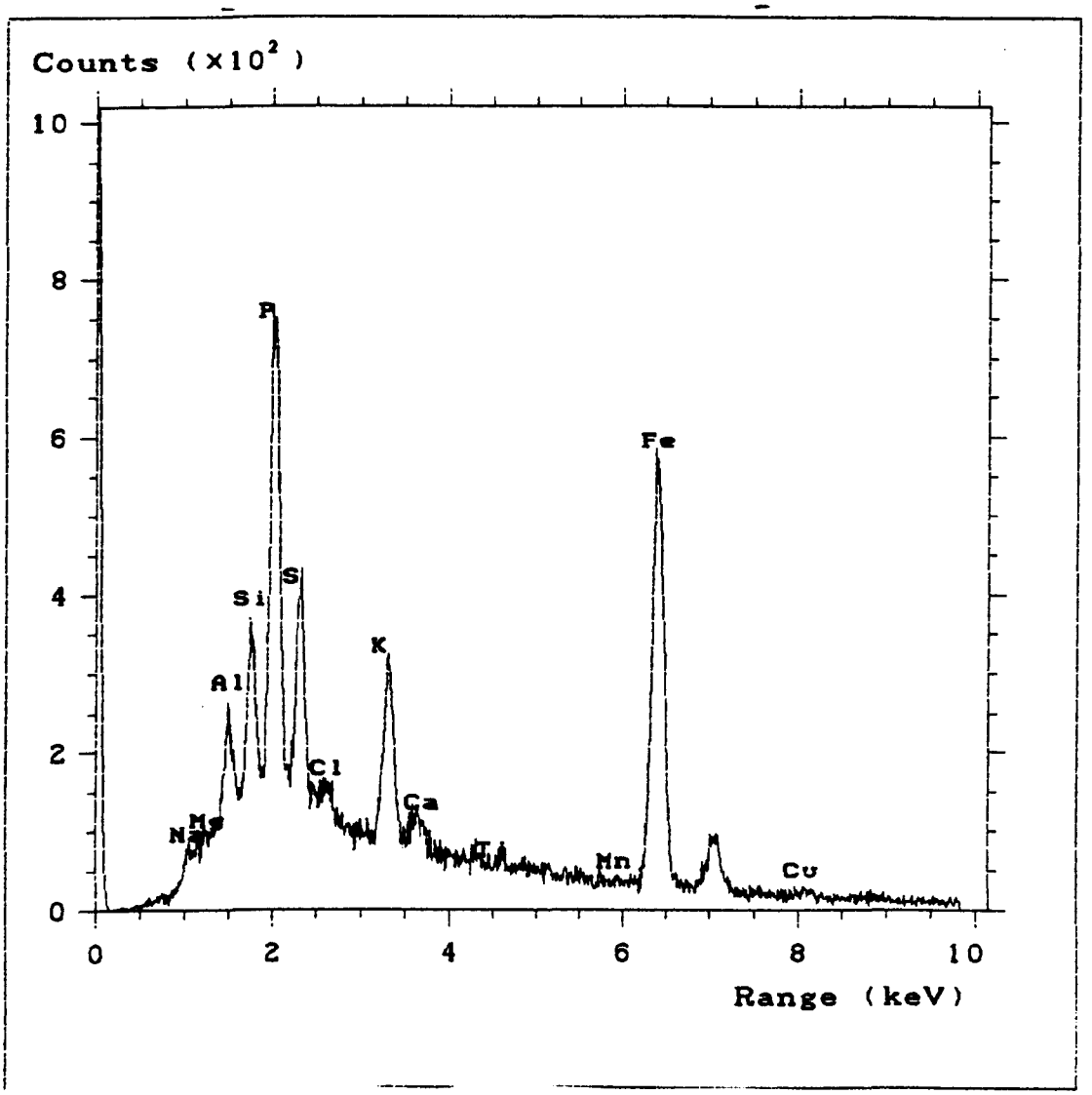


Figure 6.8. Composition of plaque deposits on roots of *P. australis* grown in the laboratory at pH 6.0 when supplied with 0.5 mg l^{-1} Cu.

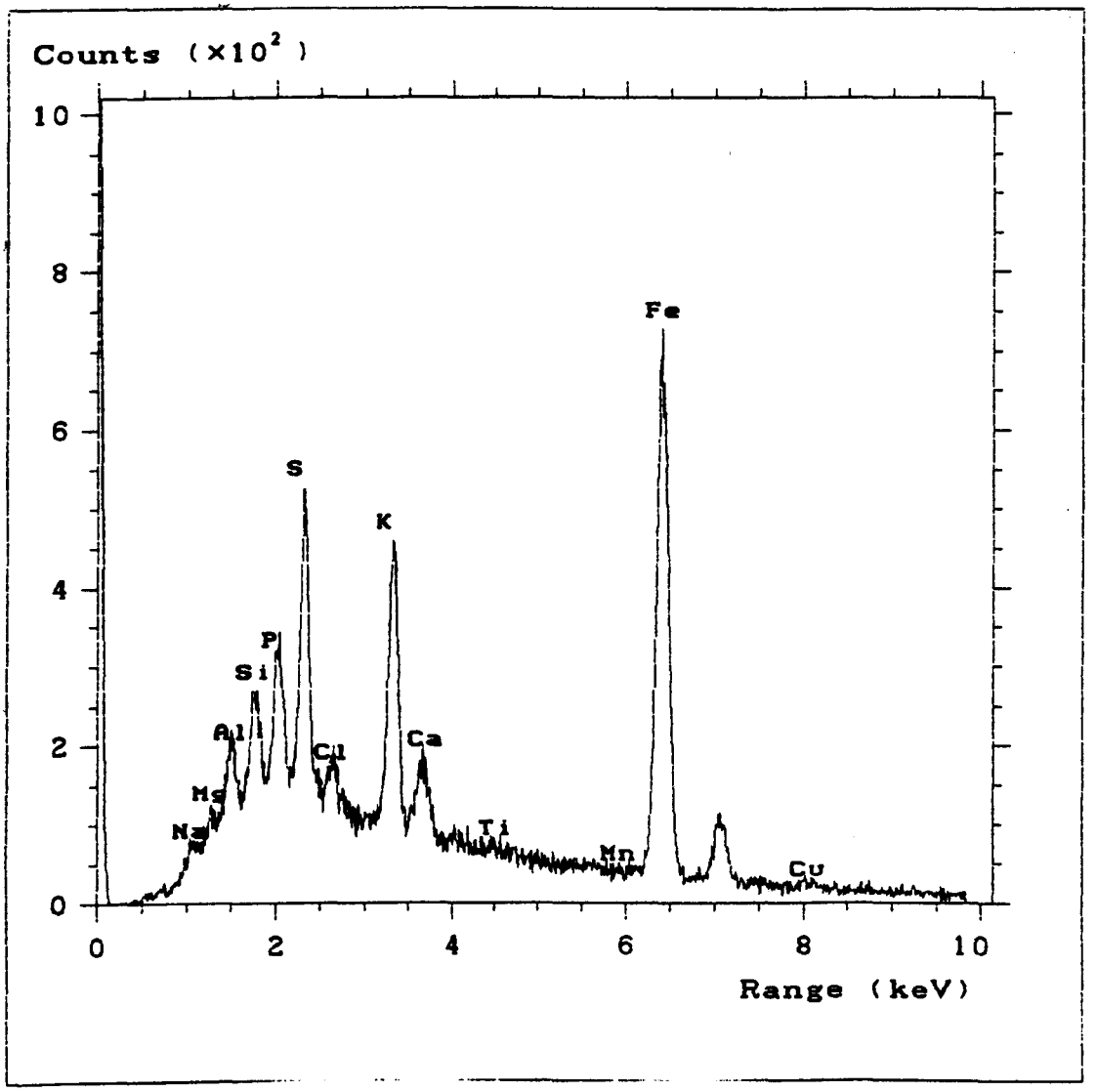


Figure 6.9. Composition of plaque deposits on roots of *P. australis* grown in the laboratory at pH 6.0 when supplied with 0.5 mg l^{-1} Mn.

6.3.3 Metal uptake in field specimens.

Woolley Colliery

	P.australis				*T.latifolia*			
	Shoot	**DCB**	**Root**	**Whole Root**	**Shoot**	**DCB**	**Root**	**Whole Root**
Fe	1974†	54836±121	1119†	55955†	19348†	64818±8281	2991†	67809†
Mn	446†	6691± 8	266†	6957†	1956†	6439±220	220†	6659†
Zn	37†	ND	15†	15†	28†	32±7	22†	55†
Al	3†	590± 0.1	ND	590†	81†	337±73	64†	400†
Cu	28.7†	ND	ND	ND	13†	13±4	2†	15†
P	1254†	173± 224	282†	455†	2084†	126±124	439†	565†

Table 6.1 Concentration of selected metals (mg kg^{-1}) in plants from Woolley Colliery. Means ± SE, n=3 except † n=1.

Parys Mountain

	P. australis				*E.angustifolium*			
	Shoot	**DCB**	**Root**	**Whole Root**	**Shoot**	**DCB**	**Root**	**Whole Root**
Fe	35677 ±8191	48842 ±17064	5920 ±2571	54761 ±15406	38558 ±8858	41090 ±6812	9406 ±4417	50496 ±10508
Mn	169±19	164±164	278±58	441.7 ±154	111±12	ND	28±10	28±10
Zn	378±53	2014±550	1596±451	3609±652	300±33	169±37	77±21	246±58
Al	71±16	ND	129±26	129±26	325±39	ND	202±9	202±9
Cu	70±7	164±164	167±65	331±152	205±13	ND	156±14	156±14
Pb	191±21	7795±745	182±88	2781±2681	595±89	99±99	420±84	486±79

Table 6.2 Concentration of selected metals (mg kg^{-1}) in plants from Parys Mountain. Means ± SE, n=3.

6.3.4 The effect of pH and plaque on seedlings exposed to 0.5 mg l^{-1} Mn.

Two-way ANOVA revealed no significant effects of pH, plaque or their interaction for root or shoot contents of Mn (Figure 6.10).

However, there was a significant effect of pH (ANOVA: $F=20.74$, $df=1,8$, $p<0.001$), and of plaque (ANOVA: $F=5.8$, $df=1,8$, $p<0.05$) on the concentration of Mn in the DCB extract, with a higher level of Mn recorded when plaque was absent than when present at pH 6.0. A greater concentration of Mn was measured in the DCB extract at high pH (6.0) than at a lower pH of (3.5). The presence of plaque also had a significant effect on the concentrations of Mn in whole roots (ANOVA: $F=9.97$, $df=1,8$, $p<0.05$) which were higher in roots without plaque at high pH. The whole root data also revealed lower concentrations of Mn at low pH (3.5) than at pH 6.0 (ANOVA: $F=26.9$, $df=1,8$, $p<0.001$). This is the same pattern as was found for the DCB extract data.

6.3.5 The effect of pH and plaque on seedlings exposed to 0.5 mg l^{-1} Cu

The presence of plaque had no effect on the Cu concentration of the DCB extract (Figure 6.11). However, there was a significant effect of pH on Cu concentrations in DCB with more Cu in the extract at pH 6.0 than pH 3.5 when iron plaque was absent (ANOVA: $F=7.36$, $df=1,8$, $p<0.05$).

There were significant effects of pH (ANOVA: $F=27.35$, $df=1,8$, $p<0.001$), plaque ($F= 45.44$, $df=1,8$, $p<0.001$) and their interaction between these treatments ($F=9.71$, $df=1,8$, $p<0.05$) on Cu concentrations in roots. Higher values were found when plaque was absent and at pH 6.0.

A significant effect of pH (ANOVA: $F=9.61$, $df=1,8$, $p<0.05$) and the interaction between plaque and pH ($F=7.57$, $df=1,8$, $p<0.05$) on the concentrations of Mn in the shoots was also found. Higher values were recorded for plants grown at higher pH in the absence of plaque. Copper concentration was significantly higher in whole roots when grown at pH 6 (ANOVA: $F=14.23$, $df=1,8$, $p<0.05$) and when plaque was absent ($F=6.77$, $df=1,8$, $p<0.05$).

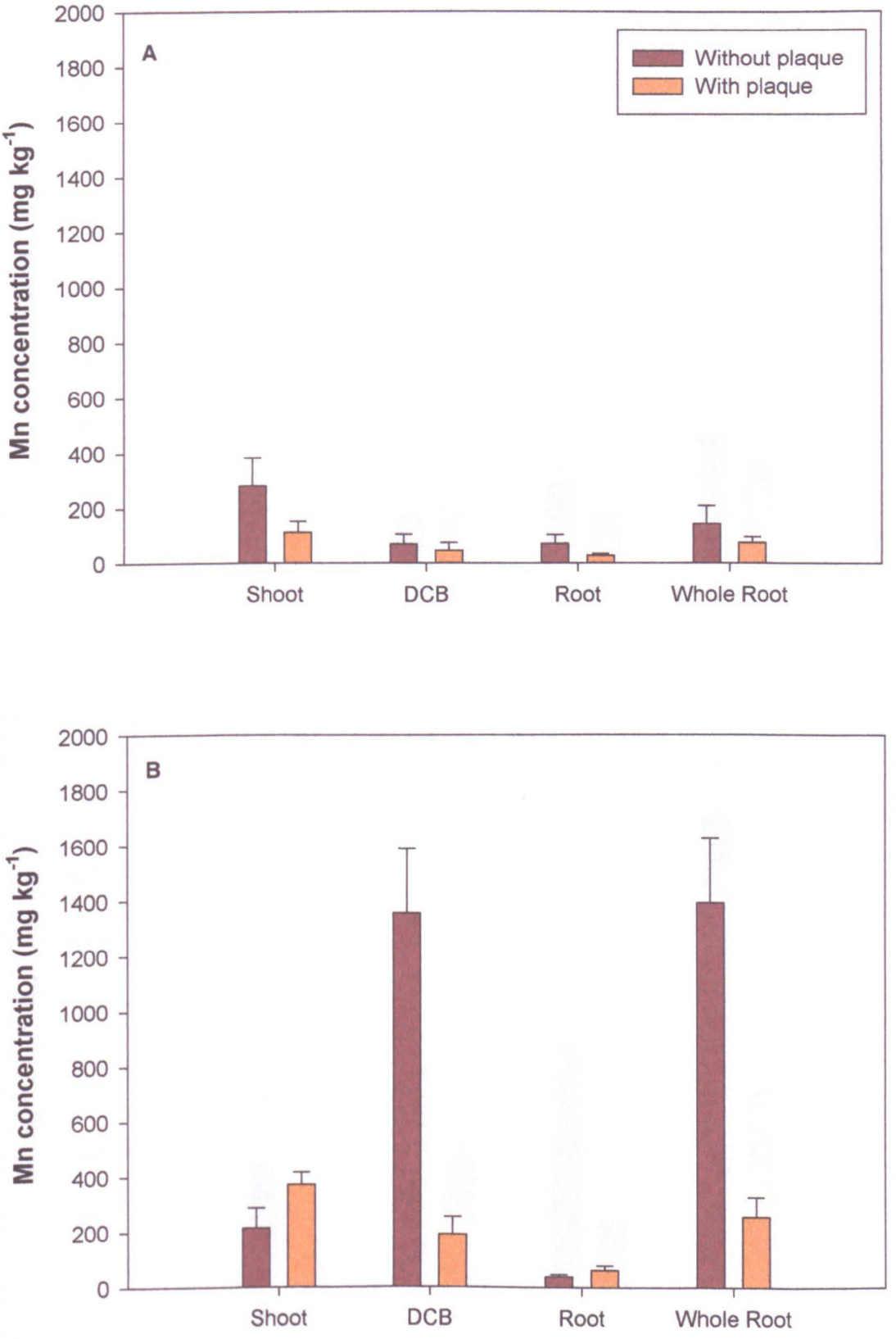


Figure 6.10. The effect of plaque on Mn concentration in *P. australis* grown in pH (A) 3.5 and (B) 6.0.

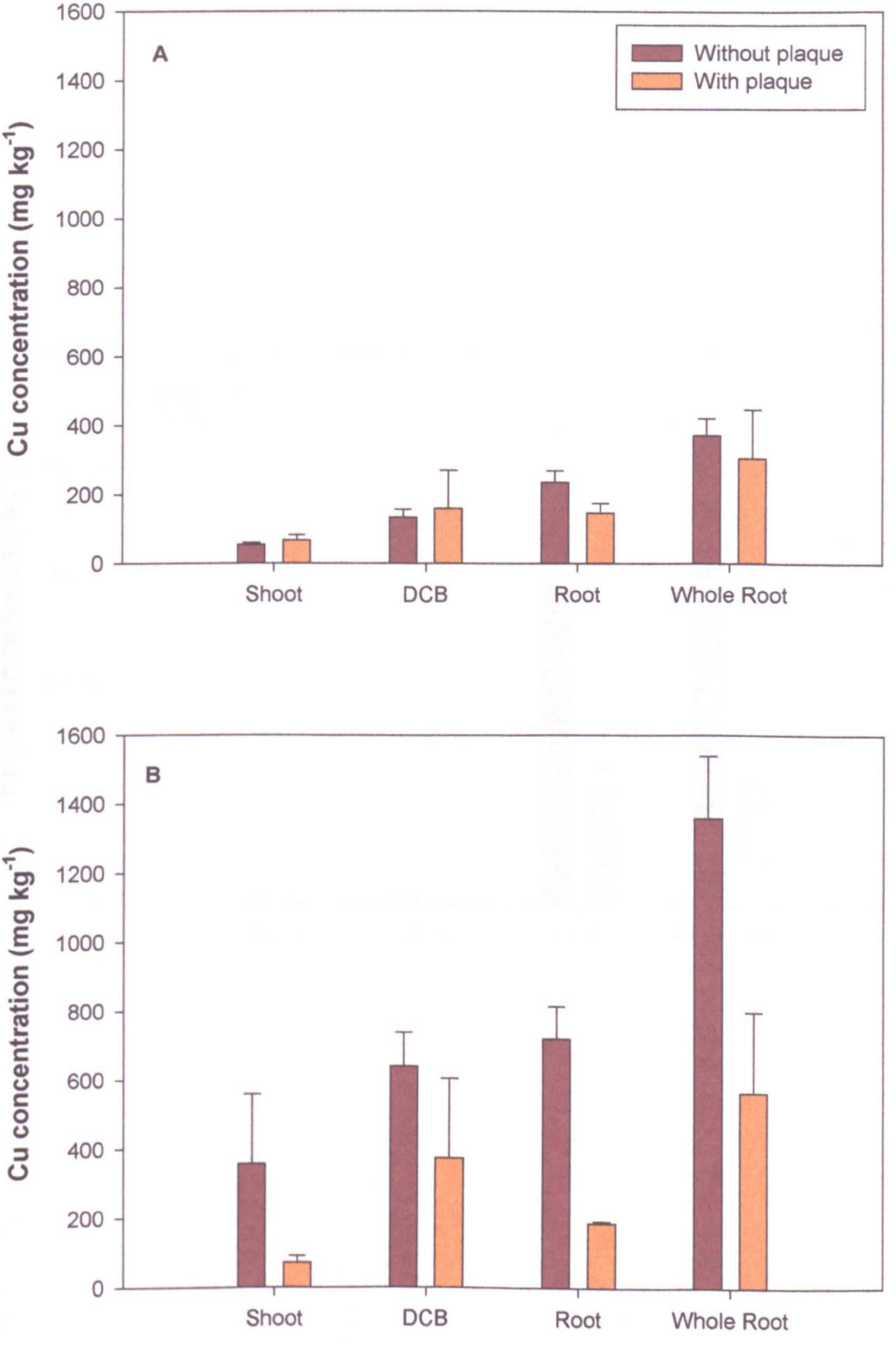


Figure 6.11 The effect of plaque on Cu concentration in *P. australis* grown in pH (A) 3.5 and (B) 6.0.

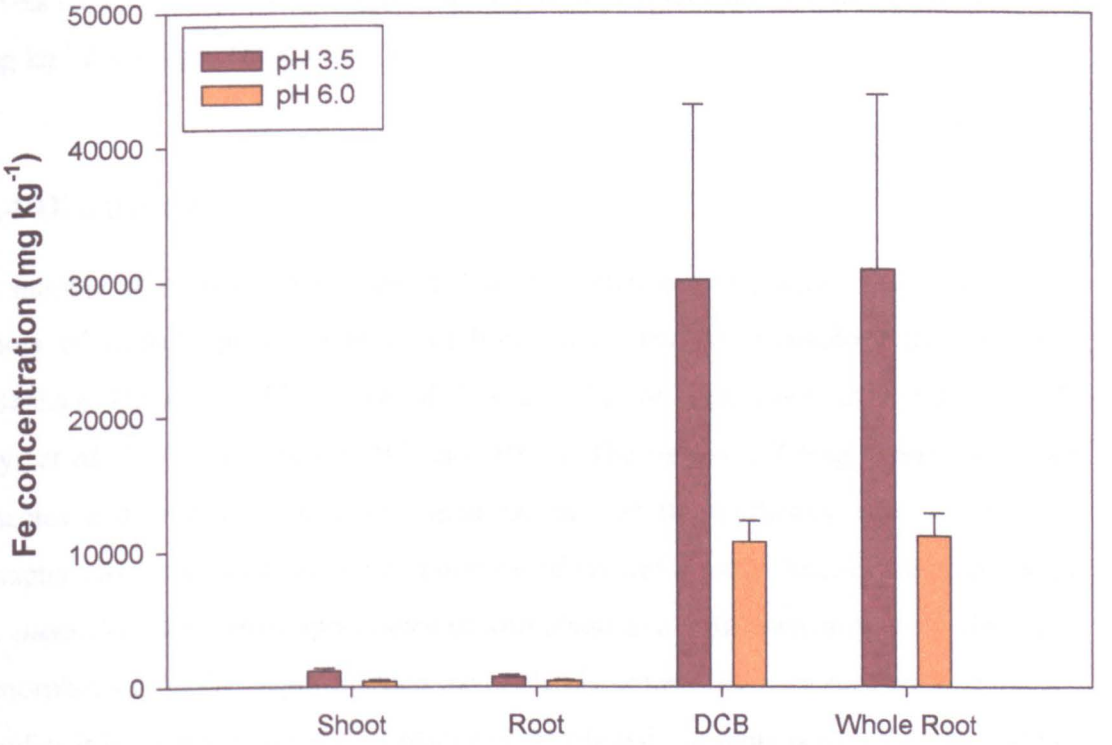


Figure 6.12 The effect of pH on Fe concentration in *P. australis*.

6.3.6 The effect of pH on seedlings exposed to 50mg l^{-1} Fe

Comparisons of Fe concentration between plants with and without plaque could not be made because those plants plaqued are supplied with a greater quantity of Fe.

There was a significantly greater concentration of Fe in the DCB extract of plants grown at pH 3.5 than at 6.0 ($t=3.04$, $df=4$, $p<0.05$). No significant difference was found in the concentration of Fe in shoots, roots and whole roots between the two pH levels ($p>0.05$). Fe concentrations in the plaque extract ranged from 10750 to 30120 mg kg^{-1} dry weight (Figure 6.12).

6.4 Discussion

A number of authors have attempted to characterise iron plaques that form on the roots of wetland plants in terms of both their chemistry/mineralogy and structure (Bacha & Hossner 1977; Chen *et al.* 1980a, b; Taylor *et al.* 1984; Otte *et al.* 1989; St Cyr *et al.* 1993; Snowden & Wheeler 1995). The use of differing techniques, plant species and laboratory and field material has led to conflicting information. This chapter has provided a unique comparison of laboratory and field-formed plaques on *P. australis*. The visual appearance of iron plaques in both environments is that of an amorphous granular deposit which incompletely covers the root surface. In previous studies it has been reported that plaque is distributed unevenly across the root surface with distinct patterns of zonation (Chen *et al.* 1980b; Taylor *et al.* 1984; Otte *et al.* 1989; Snowden & Wheeler 1995). This was also found in the present study with the majority of plaque forming in the region 1cm from the root tip. However, it has also been demonstrated that plaque distribution may vary within this region with isolated clean areas found within these heavily plaqued zones. In field roots these non-plaqued areas are less apparent probably due to the extended period of time that the field plants have been exposed to high levels of iron, more than 1 year in contrast with 6 months in the laboratory experiments.

In both field and laboratory samples there was no evidence of the formation of polyhedra or casts as reported previously (Chen *et al.* 1980b; Taylor *et al.* 1984), neither were deposits of iron found within the cells of the root, except in isolated cases. The plaques formed under laboratory and field conditions differed in their

chemistry. Under laboratory conditions the presence of iron and phosphorus indicated that the plaque was either composed of iron phosphate or as iron oxide with phosphorus adsorbed onto the surface, but this is unclear. Iron phosphate complexes have already been documented in plaques (Snowden & Wheeler 1995), however it has been suggested that $FePO_4^{2-}$ is one of the phosphate surface species that are adsorbed onto iron oxides (Kuo 1986). Iron phosphate does not form on the field specimens due to the extremely low concentrations of phosphorus in coal mine drainage waters. In these plaques the iron is associated with either S, Si and Al. This suggests that iron forms iron oxides with some elements adsorbed to the surface. The aluminium may either be adsorbed to the iron oxide surface or may form aluminium oxides, but this remains unclear. In the extremely acidic environment of Parys Mountain S is associated with the iron oxide, possible adsorbed onto the surface. In the laboratory S was not found to be associated with the iron precipitate. It has been shown that P can compete with sulphate ions for adsorption onto metal oxides and that the presence of P decreases the sorption of sulphate over the whole range of pH at which it is sorbed (Geelhoed *et al* 1997). Where phosphorus is present in the laboratory cultures this could prevent the sorption of S on to the iron oxide precipitate. However, in the field where P concentration is very low, sulphate can be sorbed on the iron plaque.

The concentration of iron in the laboratory plaques reached values which were significantly higher than previously reported in field samples of *P. australis* (St Cyr & Crowder 1988a,b, 1989; Wang & Peverly 1996). Equivalent results for laboratory samples have not been reported to date. Iron was at a greater concentration in the DCB extract at pH 3.5 than at 6.0. Increased acidity results in an increase in the solubility of Fe and other metals and therefore more iron will be available for precipitation around the root surface. This contrasts with the majority of literature which has shown that the amount of Fe extracted from plaques has a positive relationship with pH (Taylor *et al.* 1984; Macfie & Crowder 1987; St Cyr & Crowder 1989; Crowder & Coltman 1993). However, the results agree with those of St Cyr & Campbell (1996) who suggested that more plaque formed at low pH due to increased solubility of Fe and that at higher pH less Fe would be available for precipitation. This also suggests that high acidity does not affect the oxidative power of the roots.

There was no significant difference in the uptake of Fe into the shoots or roots with pH therefore although there is less plaque formed at high pH this does not result in greater uptake into the plant tissues, which supports the hypothesis that less Fe is available for both precipitation and uptake.

On the surface of roots in the laboratory supplied with Mn only, small particles were found that were composed mainly of Mn with accessory elements. The formation of Mn oxide plaques has been reported elsewhere (Levan & Riha 1986; Crowder & Coltman 1993) and these particles could represent the initial stage of Mn plaque development. However, these were very rare and it is probable that the very low concentrations (0.5 mg l^{-1}) of Mn supplied were not sufficient for the formation of a plaque. If Mn was supplied at a similar concentration to Fe (50 mg l^{-1}) it is possible that Mn plaque could be induced on the roots of *P. australis.*

A number of authors have suggested that the presence of iron plaque may act as a barrier to toxic metals (Otte *et al.* 1987, 1989; Greipsson & Crowder 1992; Greipsson 1994; Wang and Peverly 1996). This may be achieved by adsorption onto iron compounds or co-precipitation. However, the majority of investigations have shown that the presence of plaque does not impede uptake (St Cyr & Crowder 1987; Benckiser *et al.* 1984; Levan & Riha 1986; Crowder & Coltman 1993; Ye *et al.* 1997, 1998a). From the SEM analysis it is evident in both field and laboratory specimens that iron plaque has an extremely limited capacity for the adsorption of other metals such as Mn or Cu. Neither of these metals is found in association with the iron plaque except for trace amounts in isolated areas of field samples. In contrast, the analytical data show that Mn and Cu are present on the surface of the root, particularly at the higher pH (6), both with and without plaque. It has been suggested previously (Bacha & Hossner 1977; McLaughlin *et al.* 1985) that the DCB extraction technique is harsh and may remove metals from within the root. In the absence of an iron plaque coating, this 'stripping' could be accentuated due to the absence of a layer of protection. The results from the DCB extraction could therefore be overestimating the concentrations of metals and should be interpreted with great care.

This proposition is also supported by the data obtained from the field specimens which show high concentrations of Cu, Pb and Zn in the DCB extracts. The EDS traces however, do not show evidence of any Pb or Zn in the plaques, and only very

small isolated examples of Cu. In addition PO_4^{2-} concentrations are higher in the DCB extracts from both *P. australis* and *T. latifolia* collected from Woolley Colliery despite the absence of this element in the EDS traces. This again suggests that the DCB extract may strip metals and other elements out from within the root tissues, thereby overestimating their concentrations.

The uptake of Mn in the shoots is lower at pH 3.5 than at 6.0 when plaque is present and for the whole roots in the presence and absence of plaque. At the lower pH there is a greater concentration of H^+ ions around the root and these can effectively compete with other metal ions including Mn^{2+}. This results in a strong inhibitory effect on the uptake of Mn (Marschner 1995). The results from the controls showed that metal uptake of trace levels of Mn was significantly less at pH 3.5 than at 6.0 for shoots, DCB extract and whole roots, which supports this hypothesis. Concentrations of Fe in whole roots and roots in the controls were also significantly lower at pH 3.5 than at 6.0.

If more Mn is taken up into the plant at higher pH and the DCB extract is harsh enough to remove metals from within the root, we would expect that there would be a higher concentration of Mn in the plaque extract at pH 6.0, which is the case. A similar result was found for Cu although in the presence of plaque there was no significant difference between the pH conditions for all plant sections. The data from the whole roots and shoots demonstrate that the presence of plaque reduces the uptake of Mn and Cu when plants are grown under higher pH conditions, but does not prevent it. The shoot concentrations of both Mn and Cu are elevated reaching 371 mg kg^{-1} dry wt and 357 mg kg $^{-1}$ dry wt, respectively. These values are much higher than previously reported in similar studies for Cu (St Cyr & Crowder 1990; Ye *et al.* 1997), but are lower than cited for Mn (Crowder & Coltman 1993).

In low pH conditions the presence of plaque does not have a significant effect on the uptake of metals. Interference by H^+ ions at low pH probably masks any potential effects of the presence of iron plaque.

Samples of *P. australis, T. latifolia* and *E. angustifolium* collected from the field also show high concentrations of metals in the plant tissues, despite extensive plaque development on the roots. Concentrations of all metals in the roots and shoots vary

both between species and between study site and also differ from values previously reported in the literature, with concentrations recorded that are both greater and lesser (Taylor & Crowder 1983; Greipsson & Crowder 1992; Ye *et al.* 1997, 1998 a,b). This suggests that although plaque may reduce the uptake of metals in certain conditions, when supplied with extremely high levels of contaminants as in the field situations reported, uptake of metals into the plants may still be significant.

6.5 Conclusions

The mechanism by which plaque reduces the uptake of potentially phytotoxic metals may involve the adsorption of metals onto the plaque surface. Plaques formed in the laboratory were probably composed of iron phosphate or hydrated iron oxides with adsorbed P, and not pure iron hydroxide as reported elsewhere (Bacha & Hossner 1977; Chen *et al.* 1980a; St Cyr *et al.* 1993). Following the adsorption of anions such as P, the pH_{ZPC} of the oxide-anion system shifts downwards and the surface becomes less negatively charged. This has been shown to increase the affinity of phosphate enriched iron oxides for metals such as Cd and Zn (Kuo & McNeal 1984; Ghanem & Mikkelson 1988). However, the plaques formed in the laboratory did not possess any adsorbed Mn or Cu suggesting that adsorption is not the mechanism by which the plaques prevent uptake. Plaques formed in the field did not contain any P and were probably composed of an iron oxide compound which differs from that formed in laboratory conditions. However, Cu was only present on the plaque surface in extremely rare and isolated areas and this suggests that adsorption is not an important exclusion mechanism.

These results contrast with many previous findings which report the adsorption of metals onto the surface of plaques (St Cyr & Crowder 1987; Crowder & St Cyr 1991; Greipsson & Crowder 1992; Greipsson 1994; St Cyr & Campbell 1996; Doyle & Otte 1997; Ye *et al.* 1997a,b , 1998a; Sundby *et al.* 1998). However, the majority of these studies have utilised the DCB extraction technique which may overestimate the concentrations of metals present as it has been shown here that metals recorded in the DCB extract are not evident in the EDS analyses of the plaque both on plants from the field and the laboratory.

7. The effect of Mn and Fe plaque on the uptake of Cu, Zn and Al in *P. australis*.

7.1 Introduction

The majority of research into plaque formation has concentrated on iron plaques (e.g. Chen *et al.* 1980 a,b; Benckiser *et al.* 1984; Greipsson 1994; Ye *et al.* 1998a). However, manganese has also been found to occur both in combination with iron plaques and also as Mn oxide deposits on the roots of wetland plants. The presence of Mn associated with Fe oxide plaques was reported on the roots of *Phragmites australis* (St Cyr & Crowder 1987, 1990) and *Vallisneria americana* (St Cyr & Campbell 1996) collected from the field. The amount of Mn found in the plaque was positively correlated with the amount of Fe (St Cyr & Crowder 1990), which was expected due to the similarity in the chemistry between the two metals. Mn plaques, as opposed to Mn associated with Fe, have also been reported to form on the roots of *Oryza sativa* from the laboratory (Bacha & Hossner 1977; Crowder & Coltman 1993). These plaques were apparent as a dark brown staining of the root. However, Mn oxides did not form if Mn was supplied at a concentration of less than 1 mg l^{-1}. In Chapter 6 the presence of isolated examples of Mn-rich particles was shown on roots of *P. australis* supplied with Mn. It was postulated that these could represent the early stages of Mn plaque formation, but as the Mn was supplied at a level of 0.5 mg l^{-1} this was probably insufficient for a complete coating of Mn oxide to form. Mn oxides have also been found on the roots of non-wetland species. Levan & Riha (1986) demonstrated the presence of Mn oxides on the roots of conifers from the field, with the ratios of Fe:Mn significantly higher than found on wetland plants. Mn oxides do not precipitate chemically below a pH of 8.6, in the studies aforementioned Mn plaques were found to form at a pH as low as 3, therefore it is more likely that Mn precipitation is biologically mediated. In SEM sections of *V. americana* roots, micro-organisms were found to be surrounded by manganese oxides and the authors suggested that these could possibly catalyse the formation of Mn oxides (St Cyr *et al.* 1993).

The presence of Mn oxide plaques on the roots of wetland plants could result in a similar role to that of iron plaques. The deposits may inhibit the uptake of other heavy metals into the plant tissues. The copper in the plaque of *P. australis* was found to be more related to the amount of Mn than to Fe (St Cyr & Crowder 1990). Mn oxides have a greater capacity for removing Cu from soils than Fe oxides (McLaren & Crawford 1973) and therefore could be more important in the immobilisation of metals. However, Levan & Riha (1986) found that Mn oxides did not prevent the uptake of Mn into the xylem sap of conifer species. It is evident that as with iron plaques, there is some confusion as to the role of Mn plaques in the control of metal uptake in plants, but they may constitute a more important mechanism than iron plaques due to the high capacity of Mn oxides for scavenging metals from the surrounding environment.

7.2 Materials and Methods

Ten uniformly-sized seedlings of *P.australis* were selected and transplanted into a blackened perspex vessel (28 x 17 x 9 cm) containing 1.5 l of 10% Rorison's solution. This was repeated for 36 units allowing for three replicates of all treatments, arranged in a randomised block design. The seedlings were grown for 53d, the Rorison's solution being changed every 3d. At the end of this period the pH of the solution in all of the containers was reduced gradually, over a period of 7d, to 3.5 using 0.1 M H_2SO_4.

The containers were split into three subsets each consisting of 12 vessels. In one of these subsets iron plaque formation was induced, in a second manganese plaque was induced and in the remaining set the roots were left unplaqued. Iron and manganese plaque formation were induced on the roots of seedlings prior to metal treatment through the addition of 50 mg l^{-1} of Fe supplied as ferrous ammonium sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$) and manganese sulphate ($MnSO_4.\ 4H_2O$), respectively, in nutrient solution which did not contain potassium phosphate, thereby preventing the interaction of Fe with P. The seedlings were placed in distilled water of the appropriate pH for 12 hrs before plaquing to prevent a similar interference of P and

were left for 7d. Plaque was visible on roots as an orange or brown deposit after 7d. All seedlings were grown for a further 5d in 10% Rorison's solution prior to metal treatment. The metals added were Cu ($CuSO_4$. $5H_2O$), Zn (ZnSO4. 7H2O) and Al ($Al_2SO_4)_3$. $16H_2O$), all at a concentration of 1.0 mg l^{-1}. These were added to the appropriate containers to give the following conditions (1) no plaque, Cu, (2) Fe plaque, Cu, (3) Mn plaque, Cu, (4) no plaque, Zn, 5) Fe plaque, Zn, (6) Mn plaque, Zn, (7) no plaque, Al, (8) Fe plaque, Al, and (9) Mn plaque, Al. Control treatments contained no additional metals. During metal exposure solutions were changed every 3d. Roots were re-plaqued at 21d and 50d to ensure coverage of roots with plaque was maintained. Prior to the repeated exposure of roots to iron or manganese, the seedlings were placed in distilled water for 6h before exposure to 50 mg l^{-1} Fe or Mn for 3d.

At the end of the experiment all plants were harvested and divided into roots and shoots. A representative root sample from each of the treatments was isolated and prepared for SEM analysis (Chapter 2.1.4).

7.3 Results

7.3.1 Morphology of plaques

Iron plaques

P. australis roots showed normal growth with good root development (Plate 7.1). Plaques were clearly visible on the root surfaces as an orange-brown colour starting approximately 1cm behind the root tip and gradually darkening with distance from this region. Under the SEM the deposits were present as a non-crystalline particulate coating of the roots surface which followed the contours of the cells (Plate 7.2). This was extensive in its coverage but areas were evident where there was no plaque deposit (Plate 7.3). In these regions the epidermal cells were clearly visible, with no evidence of damage. The plaque deposits did not form cell casts or polyhedra and did not penetrate into the cell cavities, but were an external deposit only. There was good development of root hairs over the surface of the sections and these were often coated with the particulate matter (Plate 7.4).

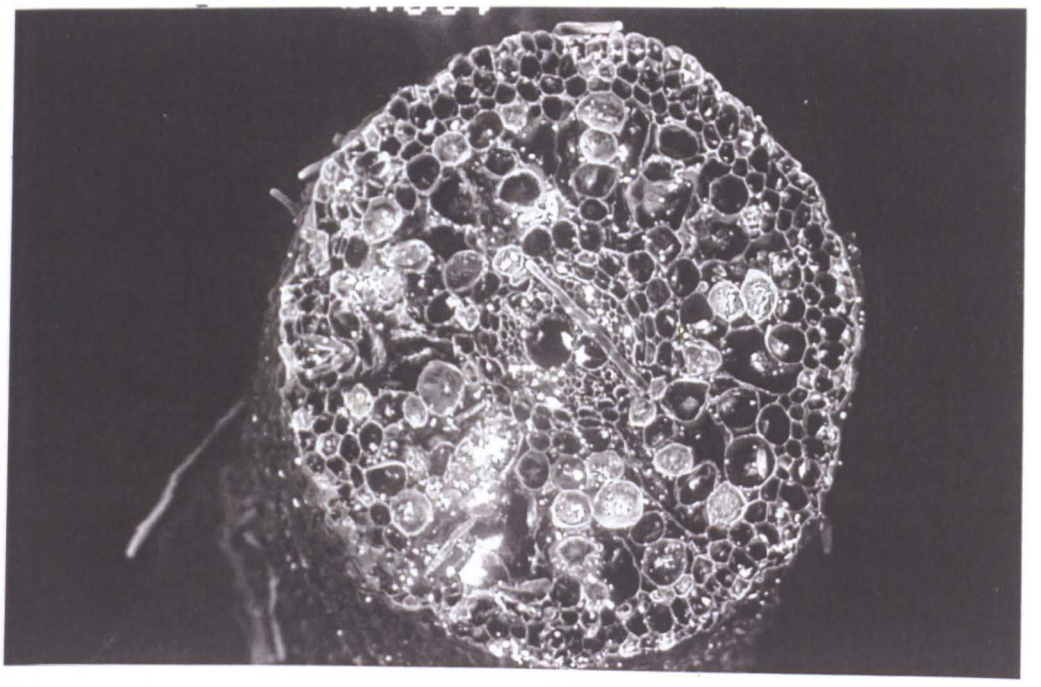


Plate 7.1. Cross section of a *P. australis* root grown in the laboratory at pH 6.0 (Backscatter image x 150).

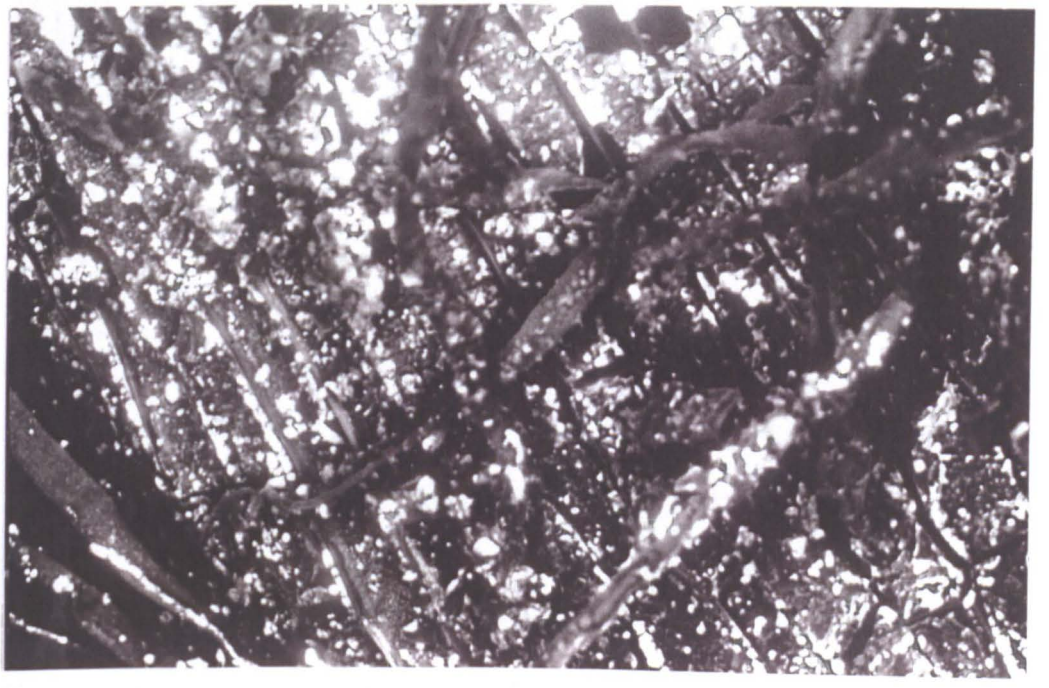


Plate 7.2. Plaque deposits on roots of *P. australis* grown in the laboratory at pH 3.5 when supplied with 50 mg l^{-1} Fe (Backscatter image x 500).

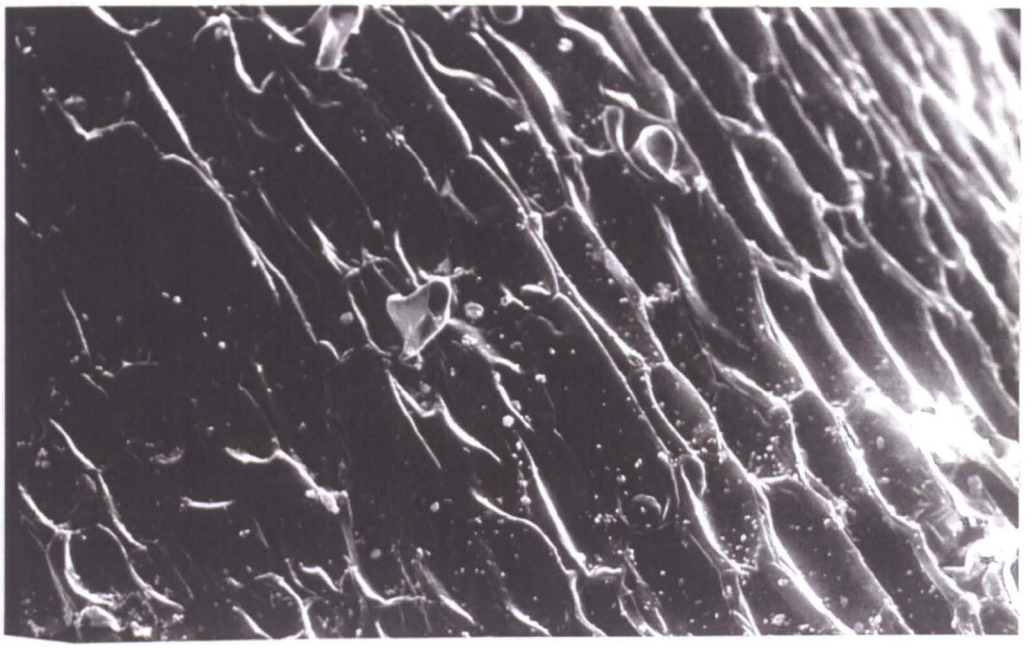


Plate 7.3. Unplaqued area on roots of *P. australis* grown in the laboratory at pH 3.5 when supplied with 50 mg l^{-1} Fe (x 450).

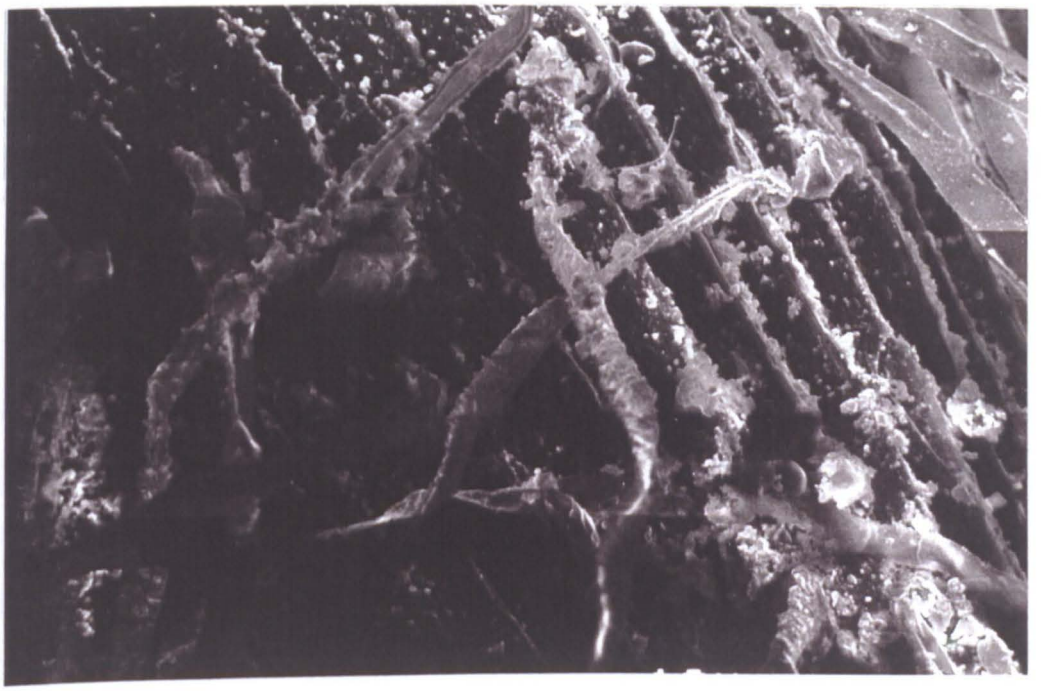


Plate 7.4. Plaque deposits on root hairs of *P. australis* grown in the laboratory at pH 3.5 when supplied with 50 mg l^{-1} Fe (x 500).

Manganese plaques

Plaques were present on the roots as a dark brown staining, but the distribution of the plaque was not as clearly defined as the iron plaques. Under the SEM the plaque was present as an amorphous particulate coating which was similar in appearance to the iron deposits (Plate 7.5). However, the Mn plaques were not as extensive in their coverage with large areas of the root with no particles evident (Plate 7.6). The Mn deposits did not penetrate into the epidermal cells and were not present around the root hairs. In one example when plaques were formed in the presence of Zn, it was observed that there was a concentration of plaque deposits around the base of a branching root. No other branching roots were evident in the other samples and so this pattern was unable to be substantiated (Plate 7.7).

In the unplaqued areas of the root there was evidence of occasional particles which differed from the plaque deposits; these were probably clay particles which originated from dust contamination in the greenhouse.

The supply of Al to plants grown without Fe and Mn plaque also produced a granular deposit of a similar nature to the other two types. This was also patchy and coated both the root surface and the root hairs (Plates 7.8 & 7.9).

7.3.2 Chemical composition of plaques

No plaques

In the absence of plaque deposits the chemical signature for the roots represented the residue of the nutrient solution, composed minor traces of K, S, Cl together with Si (Figure 7.1). There was no evidence of Fe, Mn, Cu, Al or Zn although these were present in trace amounts in the nutrient solution.

In the presence of Zn, analysis of the root surface showed a similar chemistry to those roots without additional metals, but there was an extremely small trace of Zn (Figure 7.2).

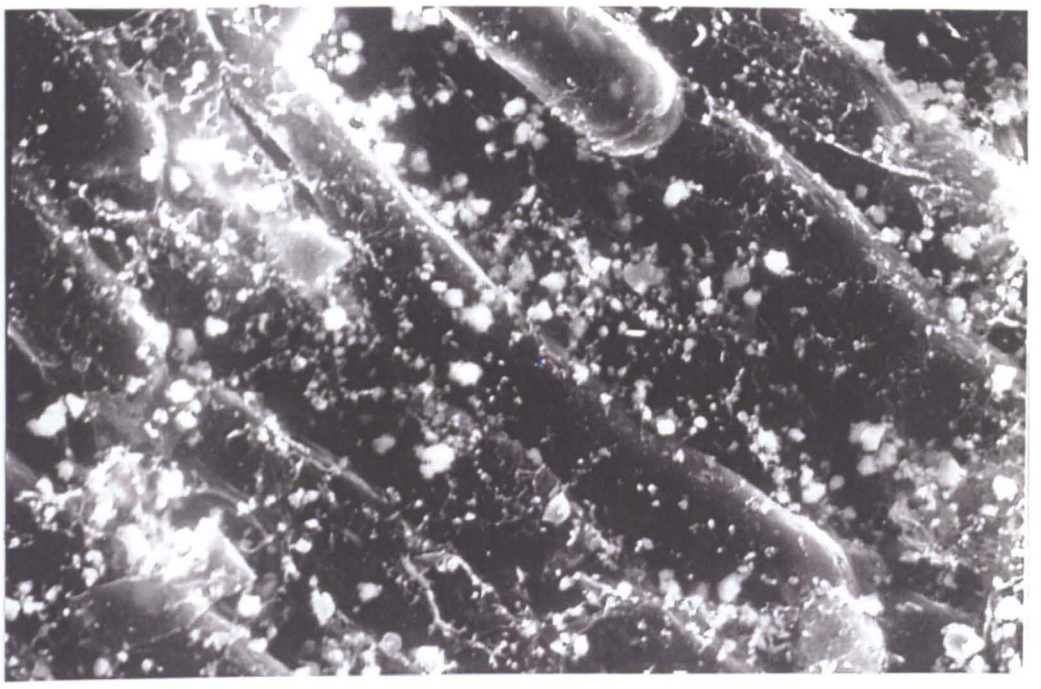


Plate 7.5. Plaque deposits on roots of *P. australis* grown in the laboratory at pH 3.5 when supplied with 50 mg l^{-1} Mn (x 1,100).

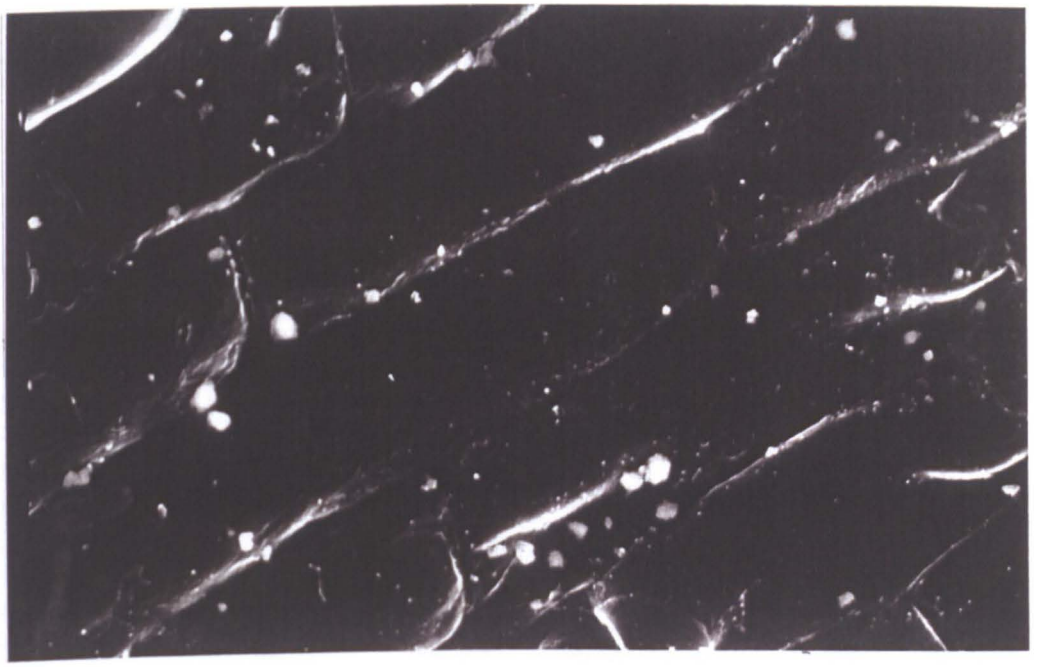


Plate 7.6. Unplaqued area on roots of *P. australis* grown in the laboratory at pH 3.5 when supplied with 50 mg l^{-1} Mn (x 1,300).

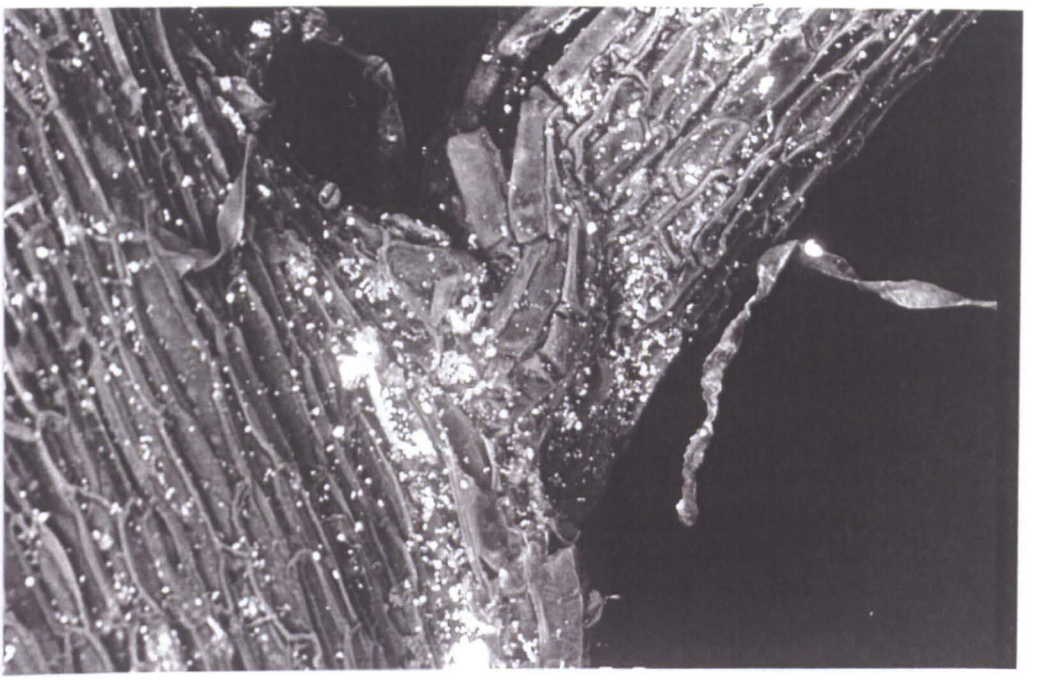


Plate 7.7. Plaque deposits at the base of a branching root of *P. australis* grown in the laboratory at pH 3.5 when supplied with 50 mg l^{-1} Mn (Backscatter image x 300).

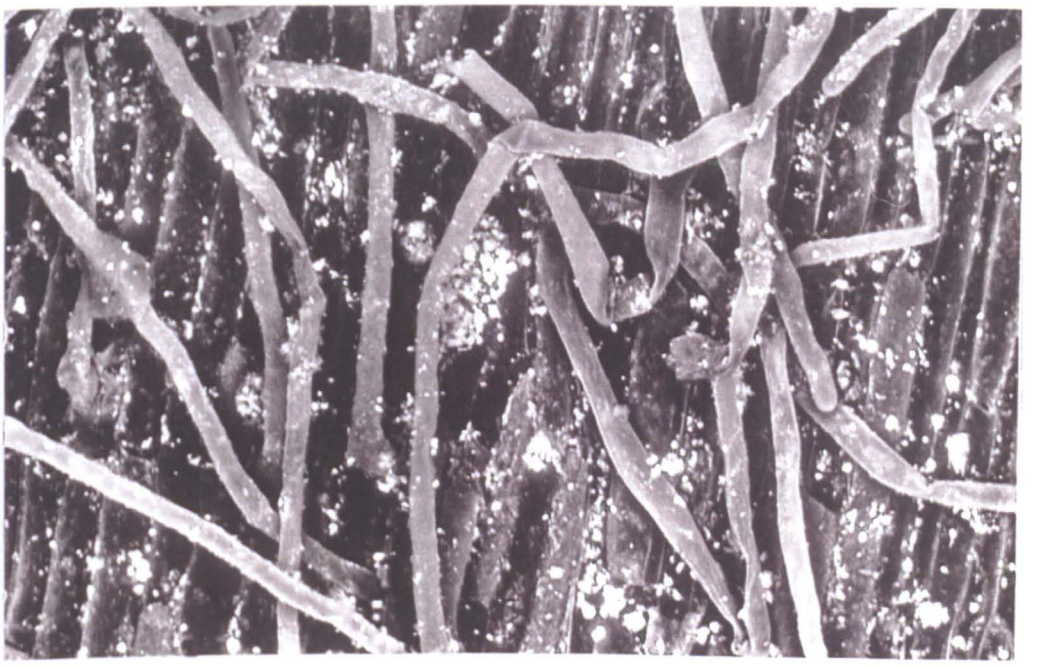


Plate 7.8. Plaque deposits on roots of *P. australis* grown in the laboratory at pH 3.5 when supplied with 1.0 mg l^{-1} Al (Backscatter image x 370).

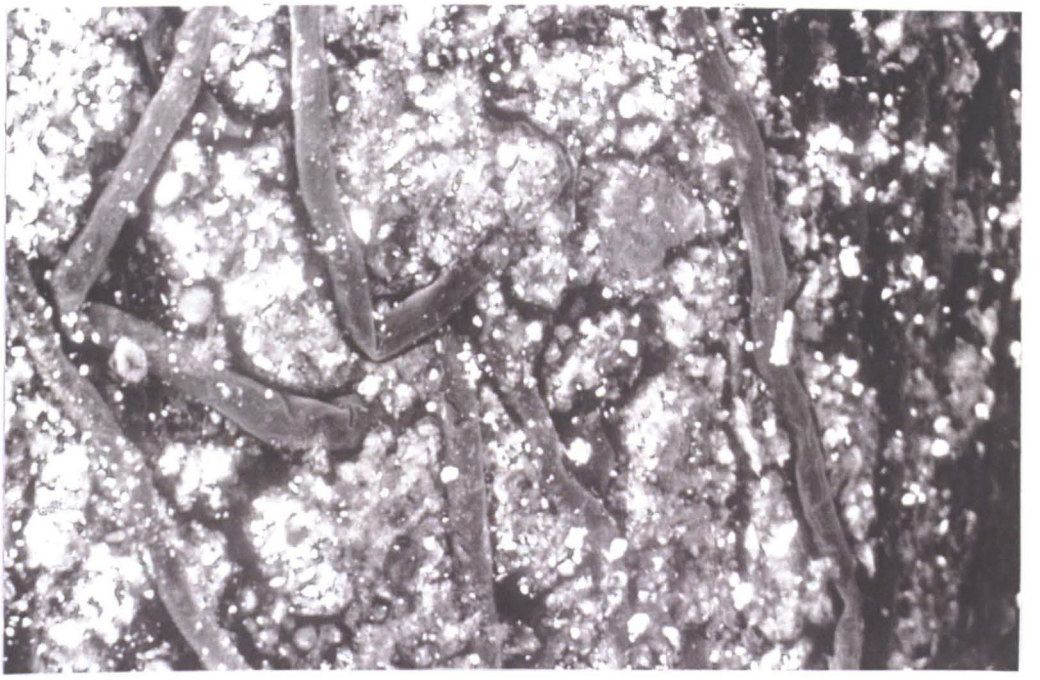


Plate 7.9. Plaque deposits on roots of *P. australis* grown in the laboratory at pH 3.5 when supplied with 1.0 mg l^{-1} Al (Backscatter image x 550).

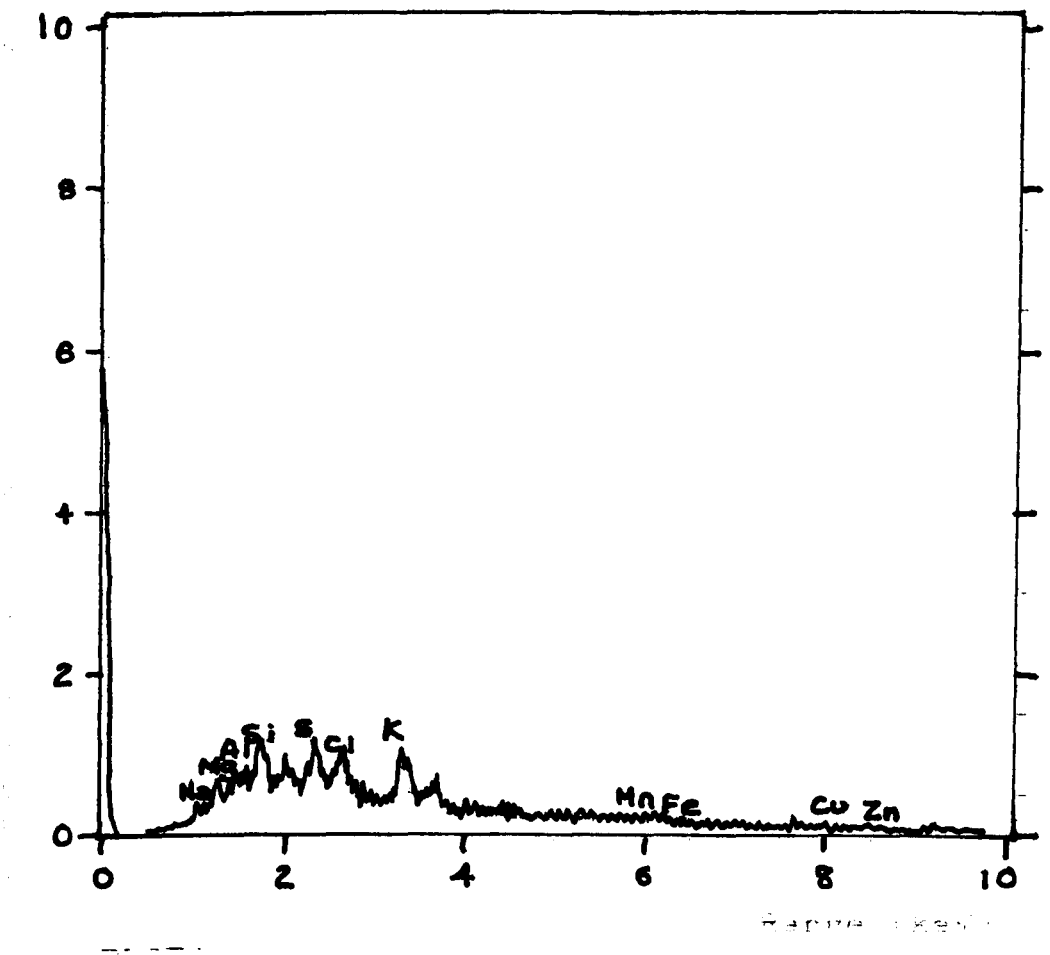


Figure 7.1. Composition of unplaqued roots of *P. australis*.

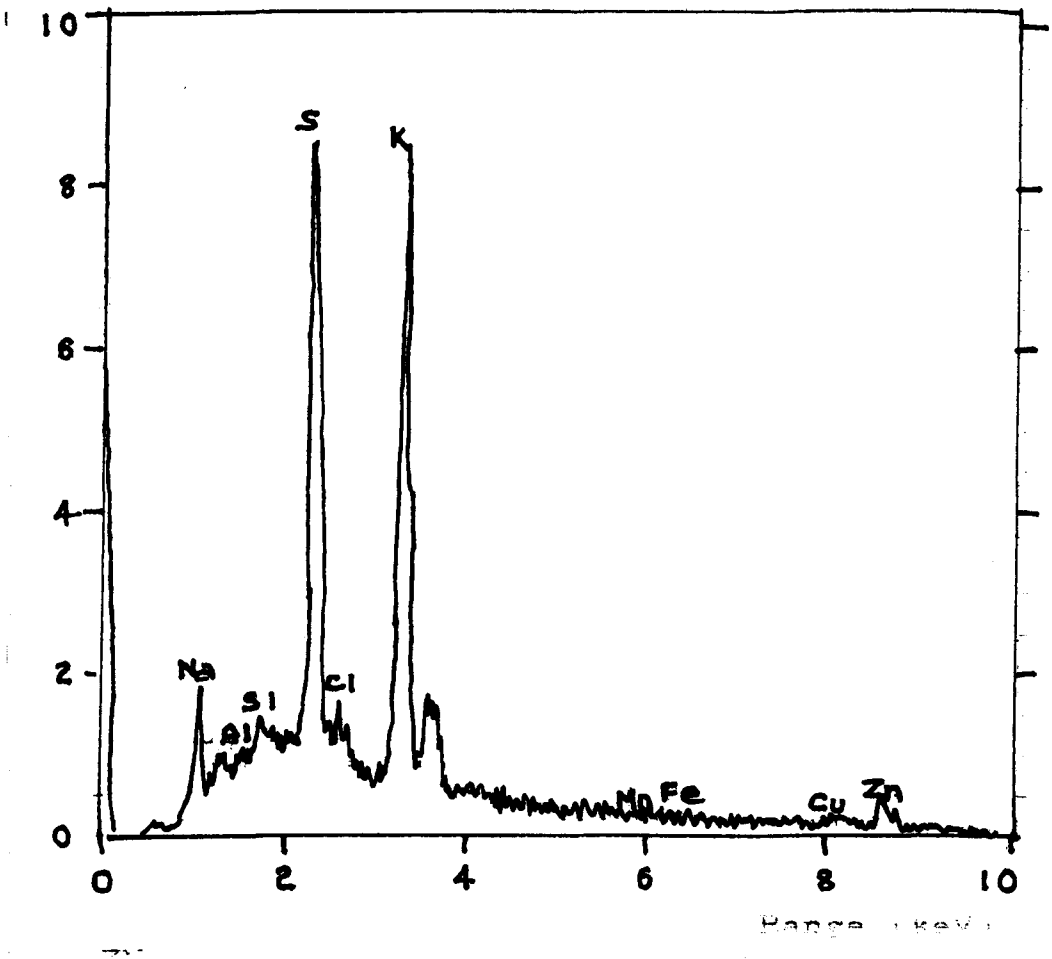


Figure 7.2. Composition of unplaqued roots of *P. australis* supplied with 1.0 mg l^{-1} Zn.

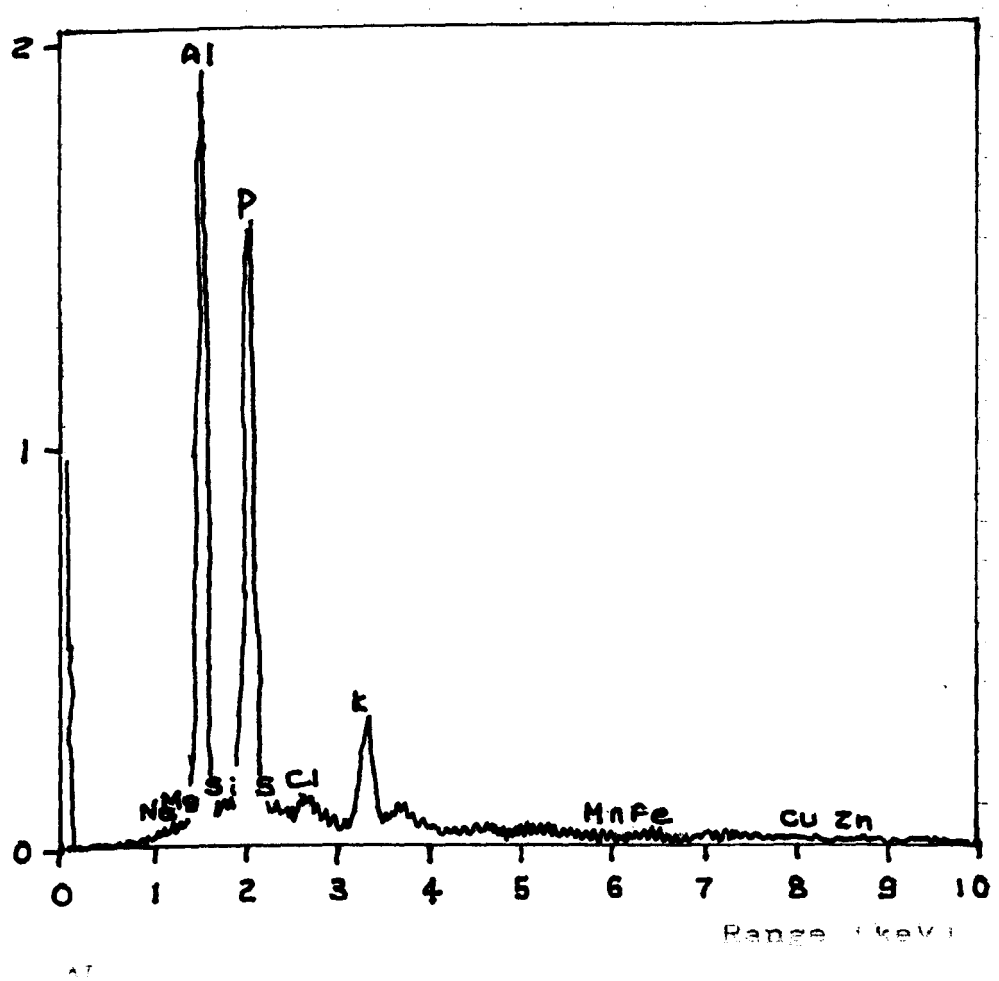


Figure 7.3. Composition of unplaqued roots of *P. australis* supplied with 1.0 mg l^{-1} Al.

When Al was supplied however, a plaque-type deposit was formed on the root surface. EDS revealed that this was composed of Al and P with minor traces of K (Figure 7.3). No Fe or Mn was associated with this plaque.

Iron plaques

The chemical signature for the deposits formed in the presence of iron only was dominated by Fe and P with no additional metals (Figure 7.4). This is the same signature that was found for iron plaques previously (Chapter 6). In the presence of aluminium, however, the deposits were formed of a combination of Fe, P and Al (Figure 7.5), which differed in their ratios with location. There was no clear pattern to this distribution. No other metals were present in this plaque deposit.

When supplied with Zn the iron plaques were composed of Fe and P, but there was no evidence for the presence of any Zn either co-precipitated or adsorbed onto the plaque (Figure 7.6). In a few regions of the root the iron plaque was formed of iron only with no evidence of P (Figure 7.7).

In the presence of copper the plaques were composed of Fe and P. No Cu was found associated with the iron plaques (Figure 7.8) .

Manganese plaques

The plaque deposits were composed of Mn with lesser amounts of K and S. This is a similar signature to that found for iron deposits with Mn replacing the iron, however they differ in that Mn deposits did not contain P (Figure 7.9). In addition, iron was always present in association with iron plaques suggesting that they precipitate out in combination. The small particles found in the clean areas of the root were found to be composed of Si and Al supporting the hypothesis that they were indeed clay particles.

In the presence of Al, the majority of the granular coating was formed of Al and P as found for the iron plaques (Figure 7.10). There was no evidence of any Mn or Fe

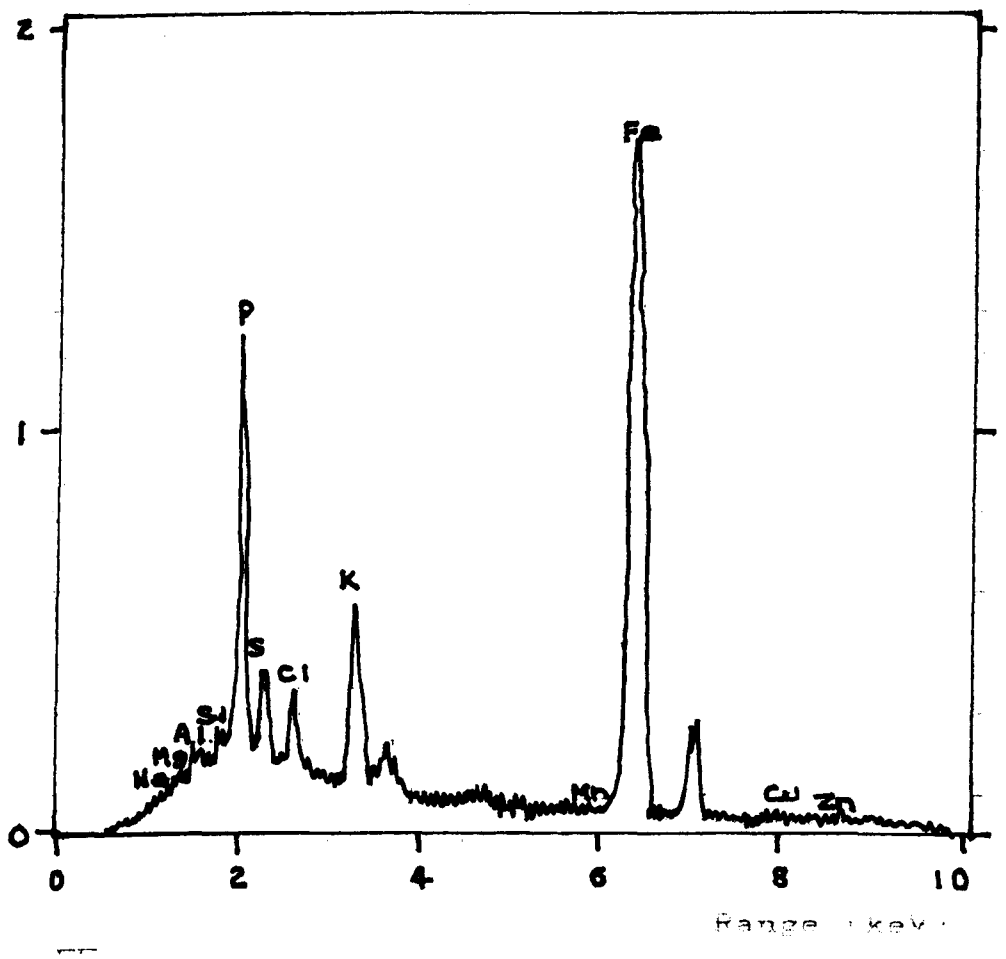


Figure 7.4. Composition of plaque deposits on roots of *P australis* supplied with 50 mg l^{-1} Fe.

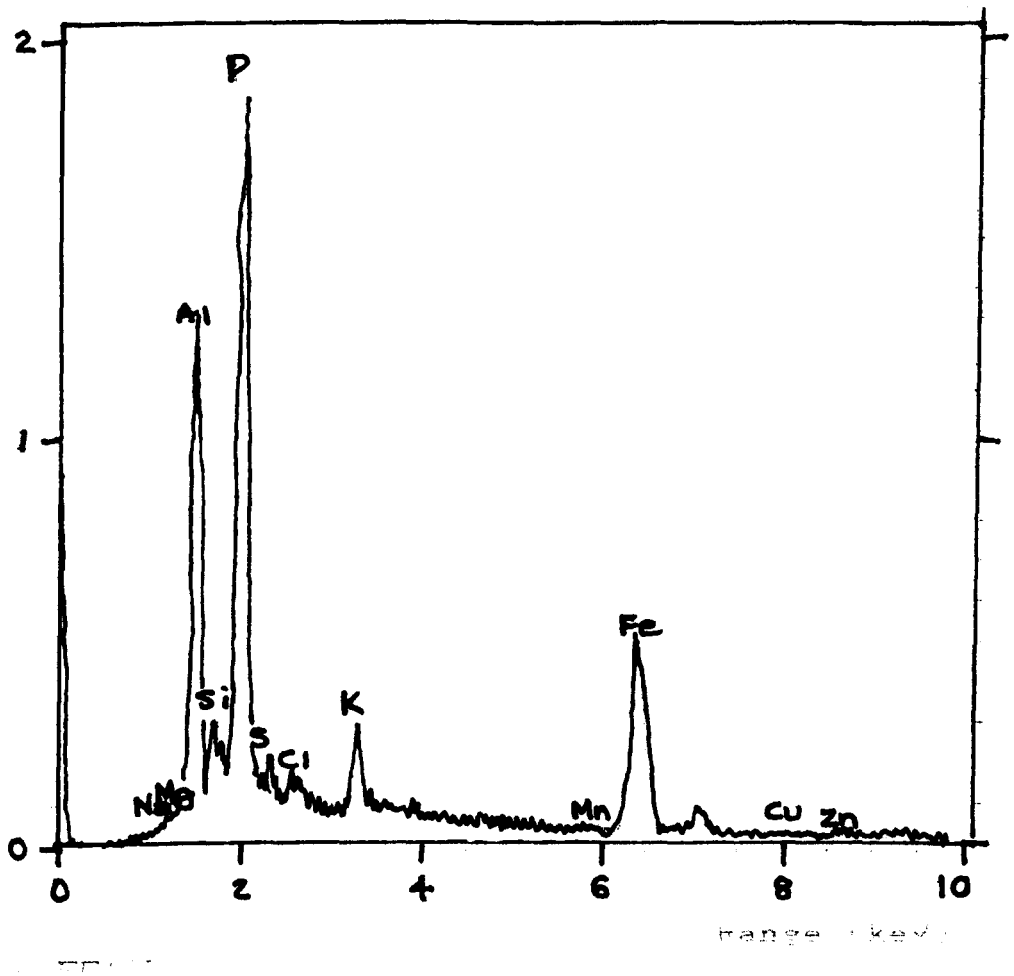


Figure 7.5. Composition of plaque deposits on roots of *P. australis* supplied with 50 mg l^{-1} Fe and 1.0 mg l^{-1} Al.

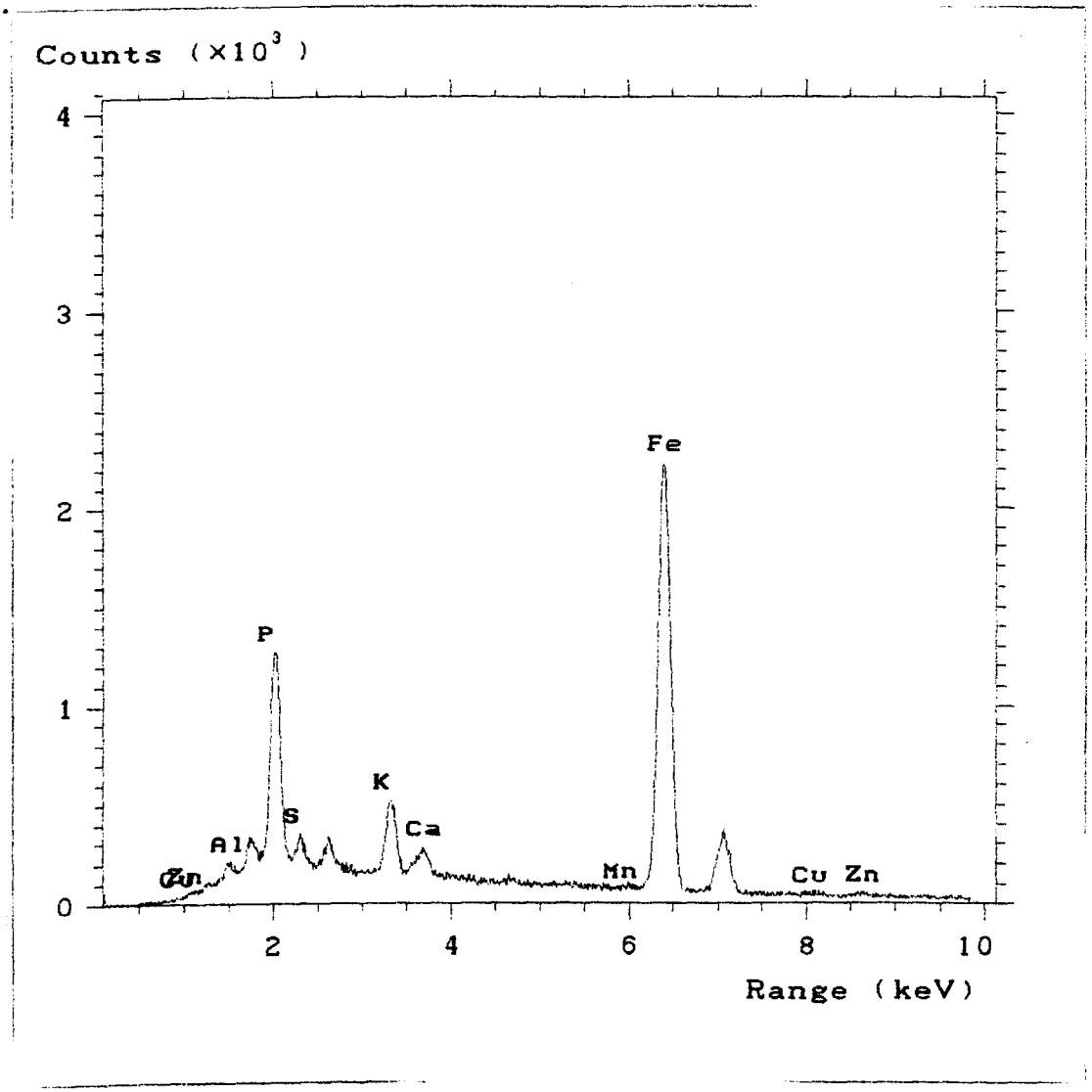


Figure 7.6. Composition of plaque deposits on roots of *P. australis* supplied with 50 mg l^{-1} Fe and 1.0 mg l^{-1} Zn.

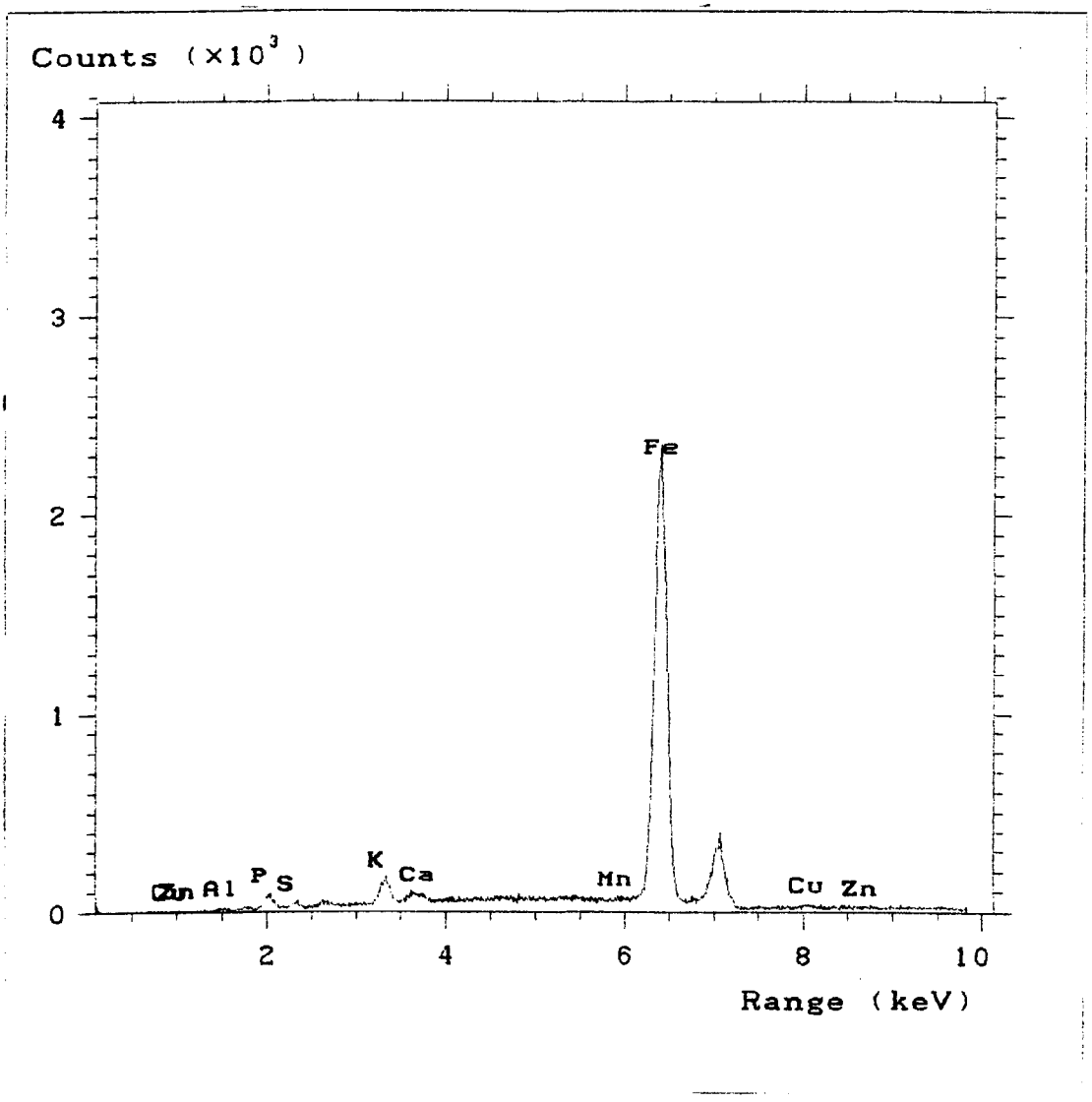


Figure 7.7. Composition of plaque deposits on roots of *P. australis* supplied with 50 mg l^{-1} Fe and 1.0 mg l^{-1} Zn.

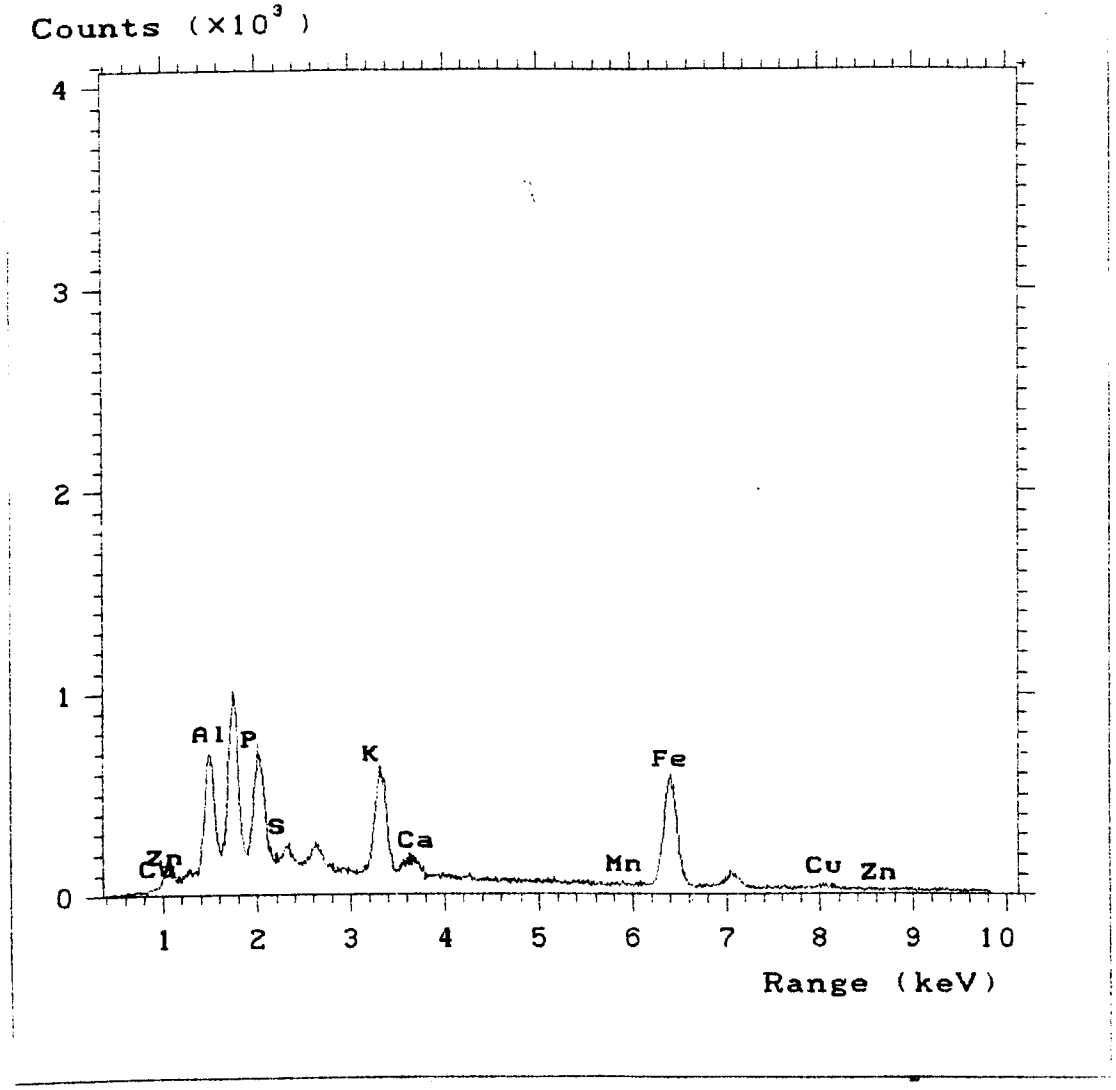


Figure 7.8. Composition of plaque deposits on roots of *P. australis* supplied with 50 mg l^{-1} Fe and 1.0 mg l^{-1} Cu.

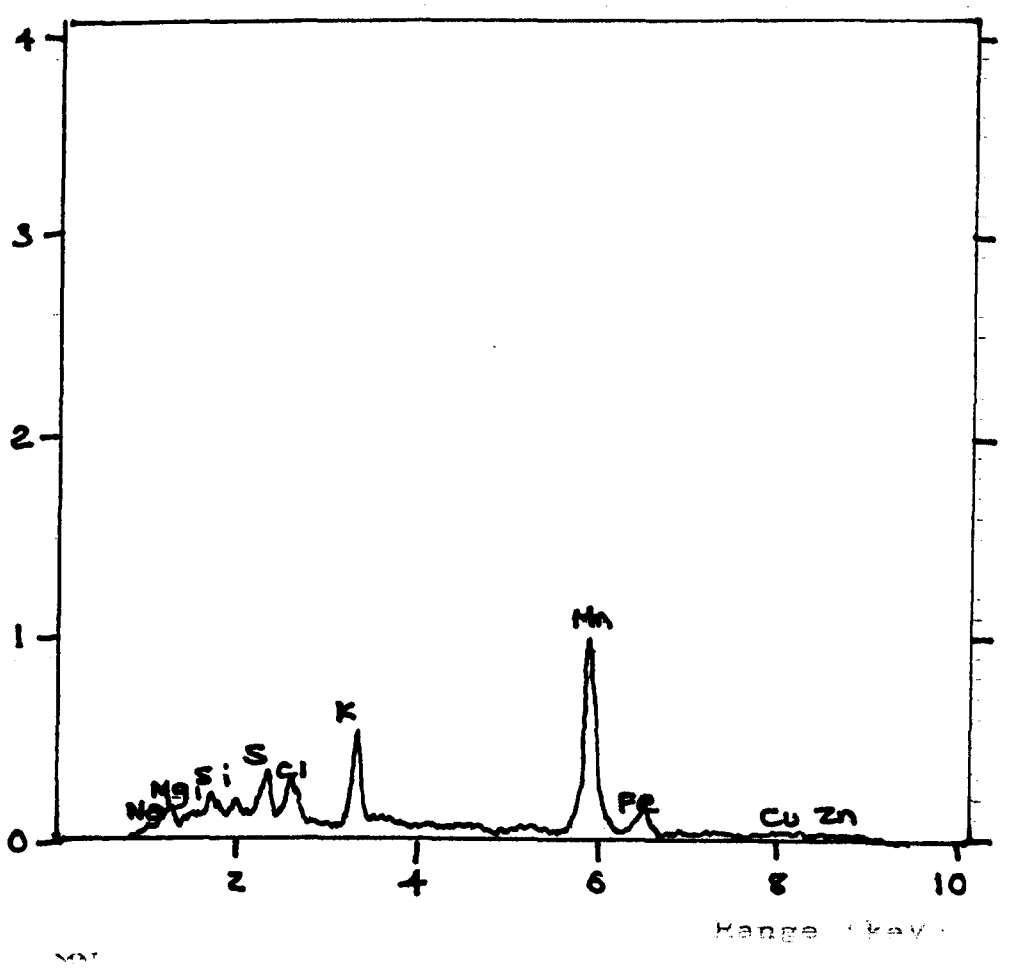


Figure 7.9. Composition of plaque deposits on roots of *P. australis* supplied with 50 mg l^{-1} Mn.

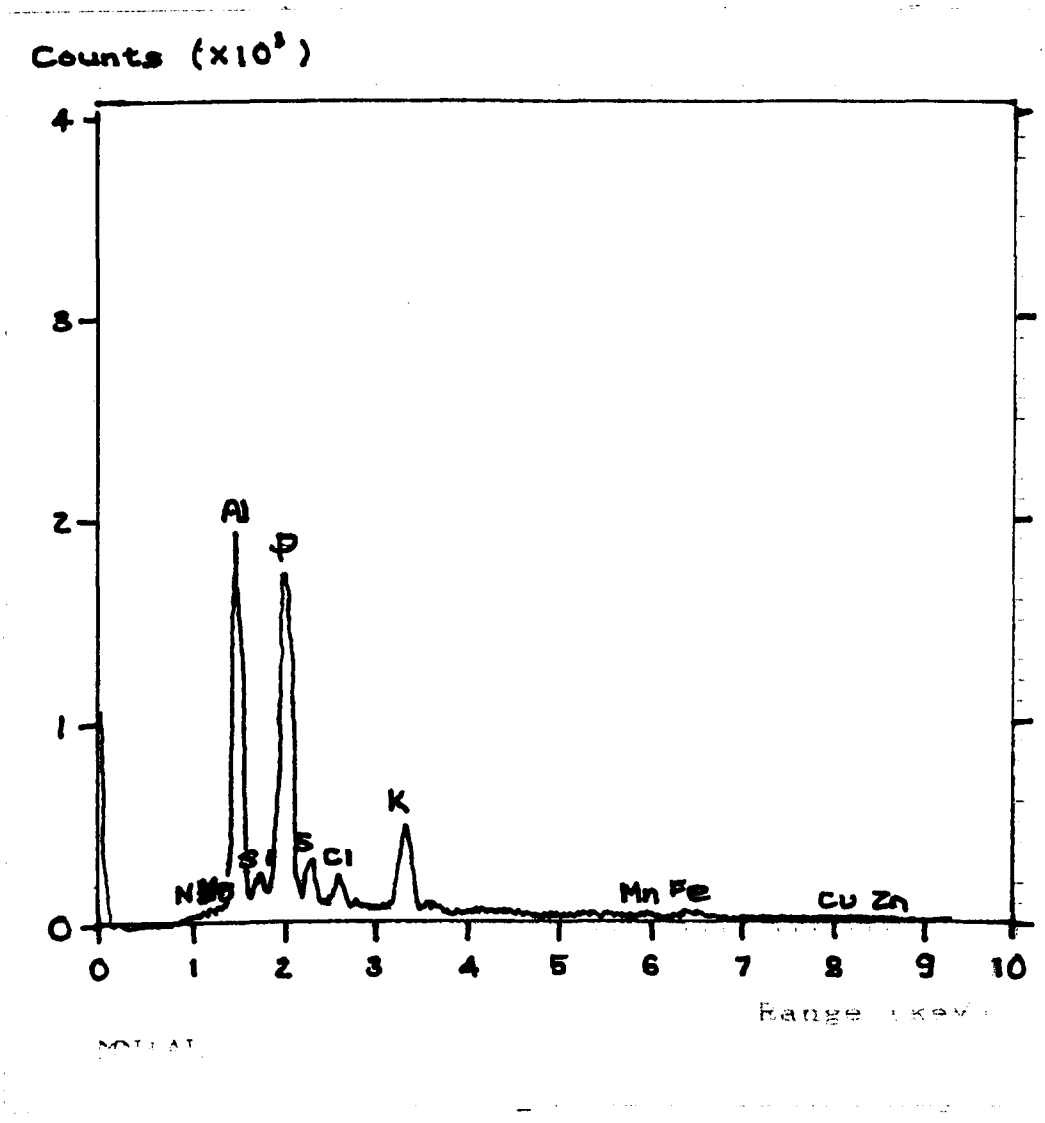


Figure 7.10. Composition of plaque deposits on roots of *P. australis* supplied with 50 mg l^{-1} Mn and 1.0 mg l^{-1} Al.

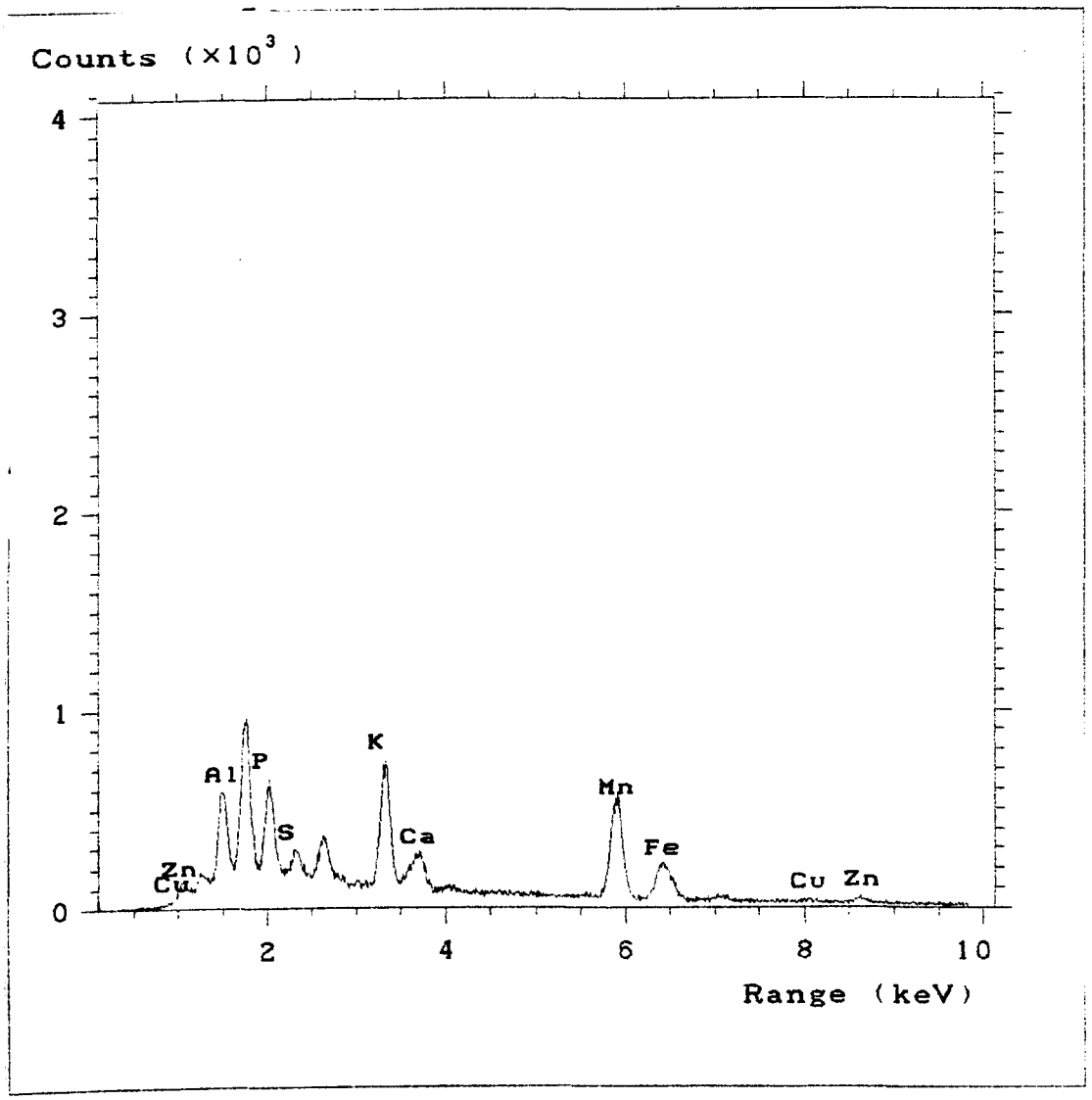


Figure 7.11. Composition of plaque deposits on roots of *P. australis* supplied with 50 mg l^{-1} Mn and 1.0 mg l^{-1} Zn.

associated with this material. Roots grown in Cu were found to possess plaques that had a chemical signature identical to those formed with Mn alone, with no count recorded for Cu. Plaques formed when Zn was added were characterised by high counts for Mn, Fe and P but in contrast with the iron plaques, small amounts of Zn were found in association with the manganese plaques (Figure 7.11).

7.3.3 The Effect of Plaque on Seedlings Exposed to 1.0mg l^{-1} Cu

There was no significant effect of plaque on the concentration of Cu in the DCB or whole roots ($p>0.05$). However, plaque had a highly significant effect on Cu concentrations in shoots ($F=11.23$, $df=2,6$, $p<0.01$) with lower concentrations found in plants with Fe plaque and without plaque than with Mn plaque. In addition plaque significantly affected copper uptake into the roots ($F=5.73$, $df=2,6$, $p<0.05$), lower concentrations were found in roots without plaque than with Mn plaque (Figure 7.12).

Overall there was more Cu in the shoots than in the roots or DCB extract. Concentrations ranged from 585-3239 mg kg $d.w^{-1}$, 453- 1308 mg kg $d.w^{-1}$ and 408-958 mg kg $d.w^{-1}$ for shoots, roots and DCB respectively.

No significant effect of plaque was detected on the uptake of P in the shoots, roots and whole roots. However, there was significantly less P in the DCB extract when plaque is absent than in the presence of Fe and Mn plaque ($F=12.48$, $df=2,4$, $p<0.05$).

7.3.4 The Effect of Plaque on Seedlings Exposed to 1.0mg l^{-1} Zn

No significant difference between the plaque treatments was found for Zn in the DCB extract or whole root data ($p>0.05$). There was a highly significant effect of plaque on Zn concentration in shoots (ANOVA: $F=11.93$, $df=2,6$, $p<0.01$), with a lower concentration in plants grown with Fe and Mn plaque than without plaque (Figure 7.14). A significant effect was also detected on the root Zn concentration (ANOVA: $F=11.77$, $df=2,6$, $p<0.01$), roots grown with Fe plaque had a lower Zn value than those with Mn plaque and without plaque. There is also a greater amount of Zn in the DCB extract in plants grown with Fe plaque but this is not significant.

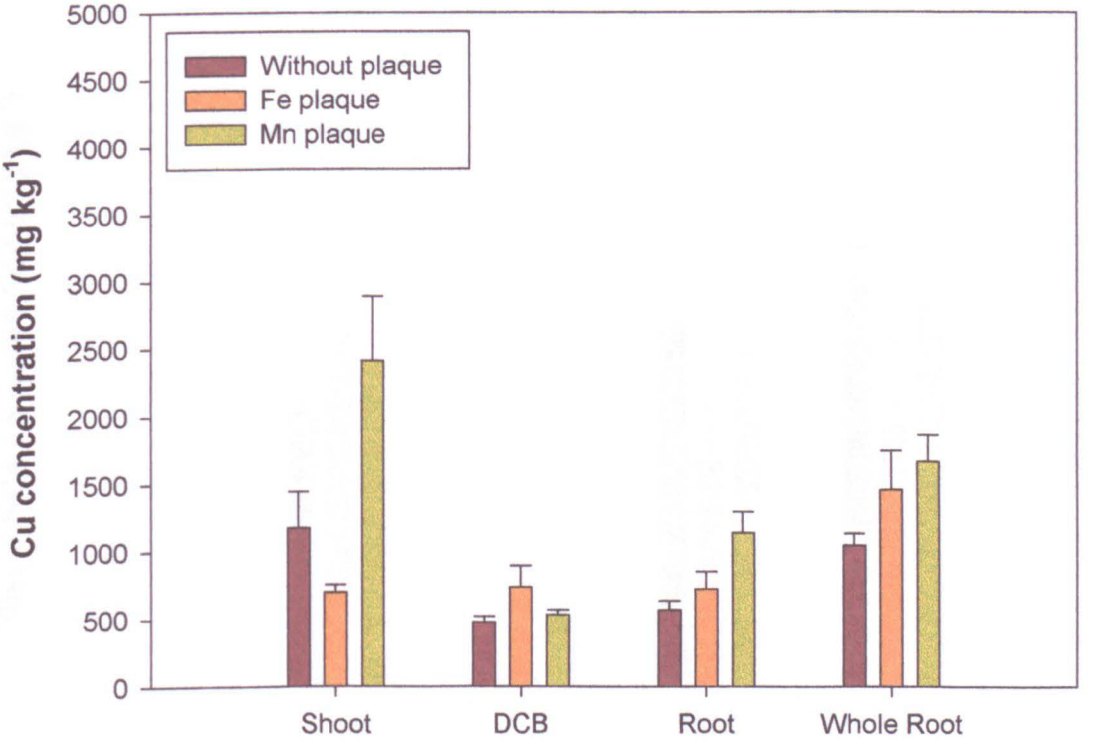


Figure 7.12. The effect of plaque on Cu concentration in *P. australis*.

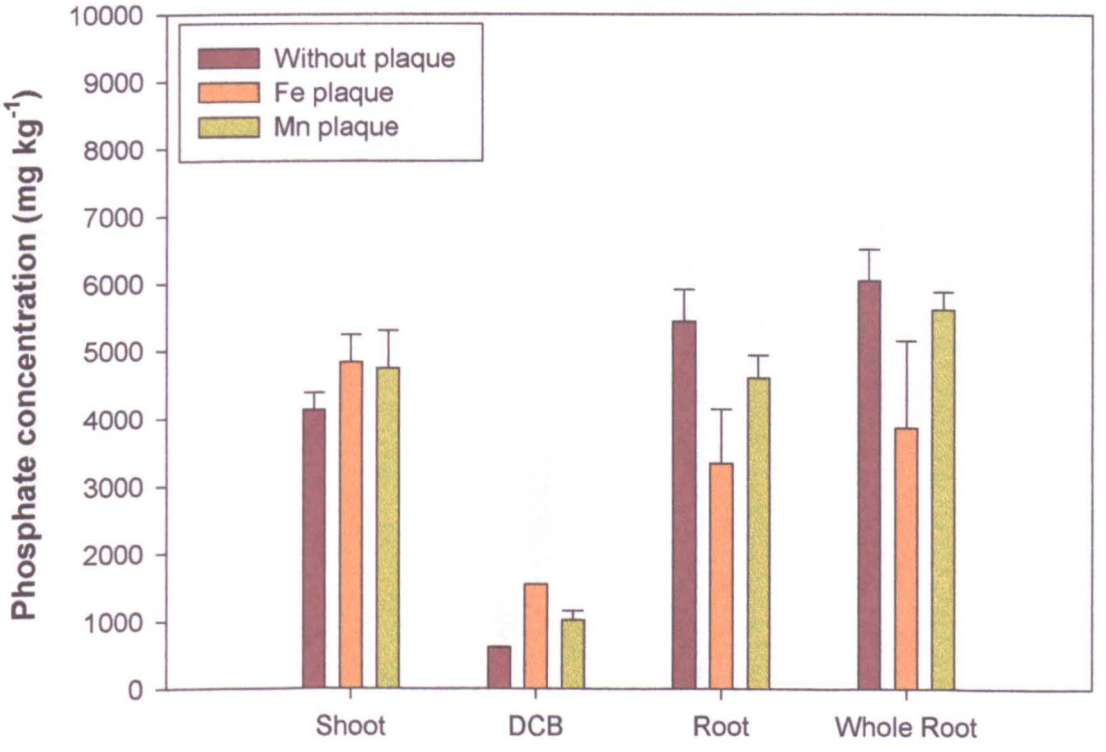


Figure 7.13. The effect of plaque on P concentration in *P. australis* supplied with 1.0 mg l^{-1} Cu.

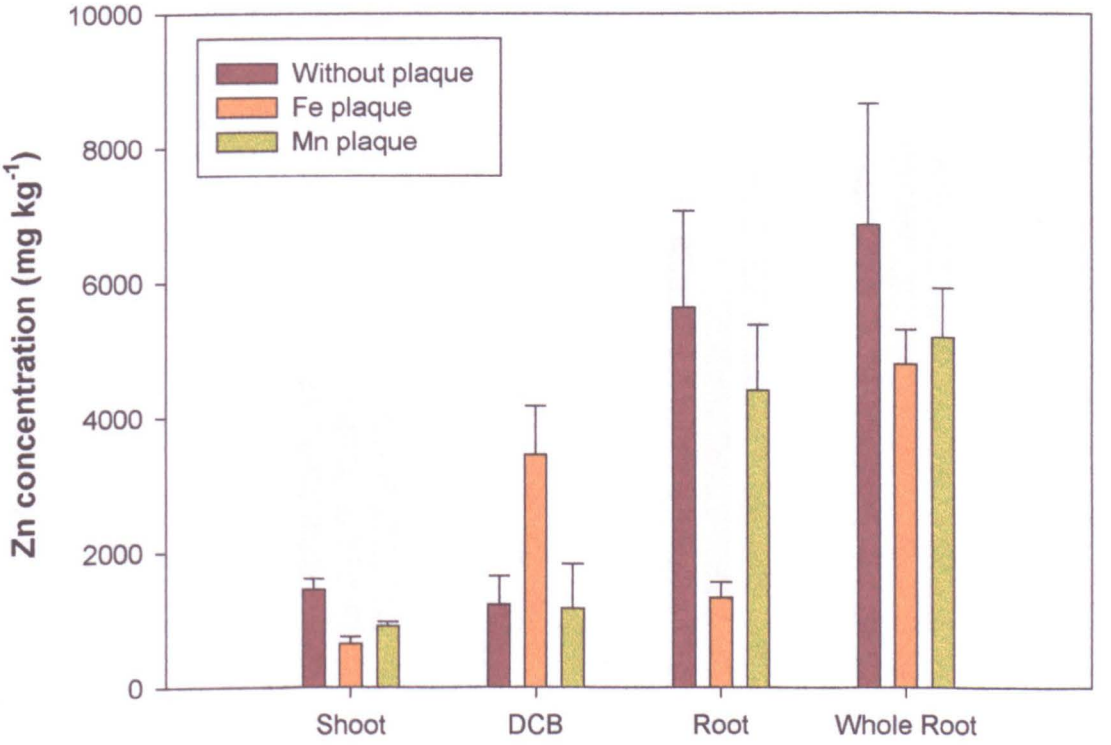


Figure 7.14. The effect of plaque on Zn concentratrion in *P. australis*.

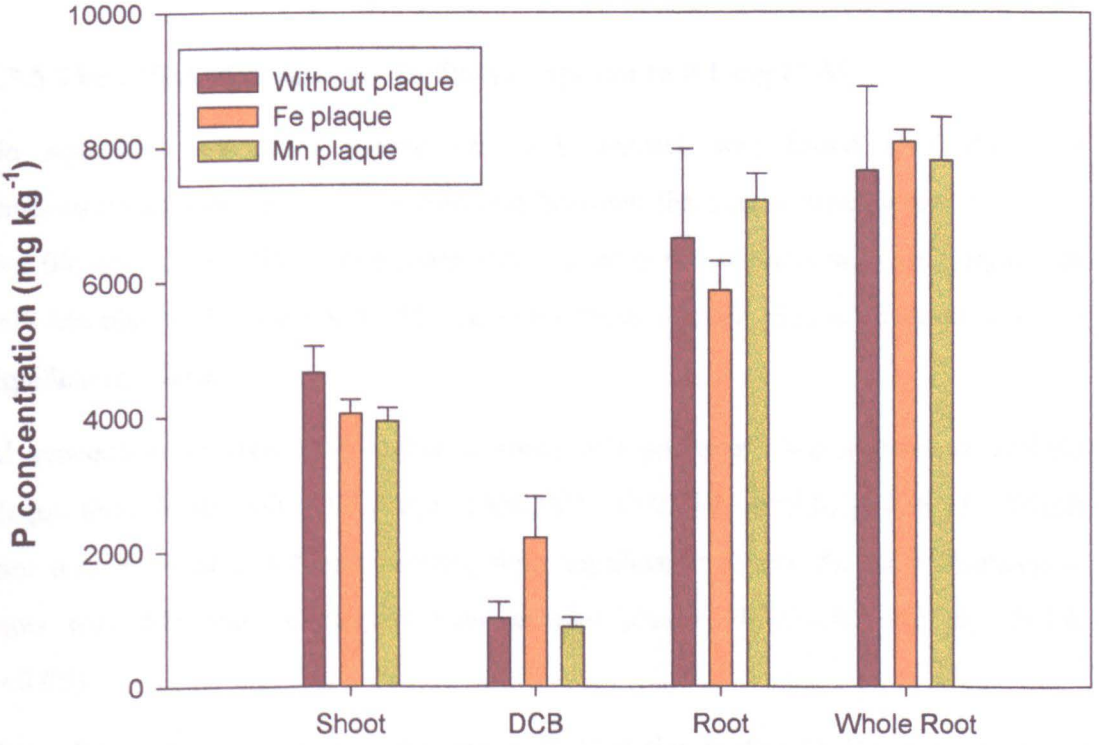


Figure 7.15. The effect of plaque on P concentration in *P. australis* supplied with 1.0 mg l^{-1} Zn.

On the whole Zn concentrations were lower in the shoots and DCB than the roots with values ranging from 445-1003 mg kg^{-1}d.w, 375-4236 mg kg^{-1} d.w and 961-8218 mg kg^{-1} d.w respectively.

The effect of plaque on P concentration in shoots, roots and DCB was not significant in all cases ($p>0.05$). However, there was less P in the plaque than in the whole roots or shoots (Figure 7.15).

7.3.5 The Effect of Plaque on Seedlings Exposed to 0.1 mg l^{-1} Al

No significant effect of plaque on DCB extract was found ($p>0.05$). Al concentrations were significantly different between the plaque treatments (ANOVA: $F=6.08$, $df=2,6$, $p<0.05$) with a lower value of Al in those plants with Fe plaque than with Mn plaque (Figure 7.16). The value for those without plaque however, was not significantly higher.

Al concentrations were also higher in roots that possessed Mn plaques or without plaque than those with a Fe plaque (ANOVA: $F=33.57$, $df=2,6$, $p<0.001$). Whole root data showed a different pattern, with significantly lower Zn concentrations in roots with Mn and Fe plaques than without plaque (ANOVA: $F=5.75$, $df=2,6$, $p<0.05$).

Generally there was a lower concentration of Al in shoots than DCB extract or whole roots in all treatments. Al values ranged from 356-965 mg kg^{-1} d.w in shoots compared to 1902-7110 mg kg^{-1} d.w for DCB extracts and 5099-14643 mg kg^{-1} d.w for whole roots.

No significant effect of plaque P values in shoots was detected. P values were however, higher in the DCB extract of roots in the presence of Fe plaque (ANOVA: $F=68.4$, $df=2,6$, $p<0.001$). This pattern was reversed in the root data, with lower concentrations of P found in roots which had Fe plaque (ANOVA: $F=13.96$, $df=2,6$, $p<0.01$). Overall there was less P in shoots and DCB extract than roots or whole roots (Figure 7.17).

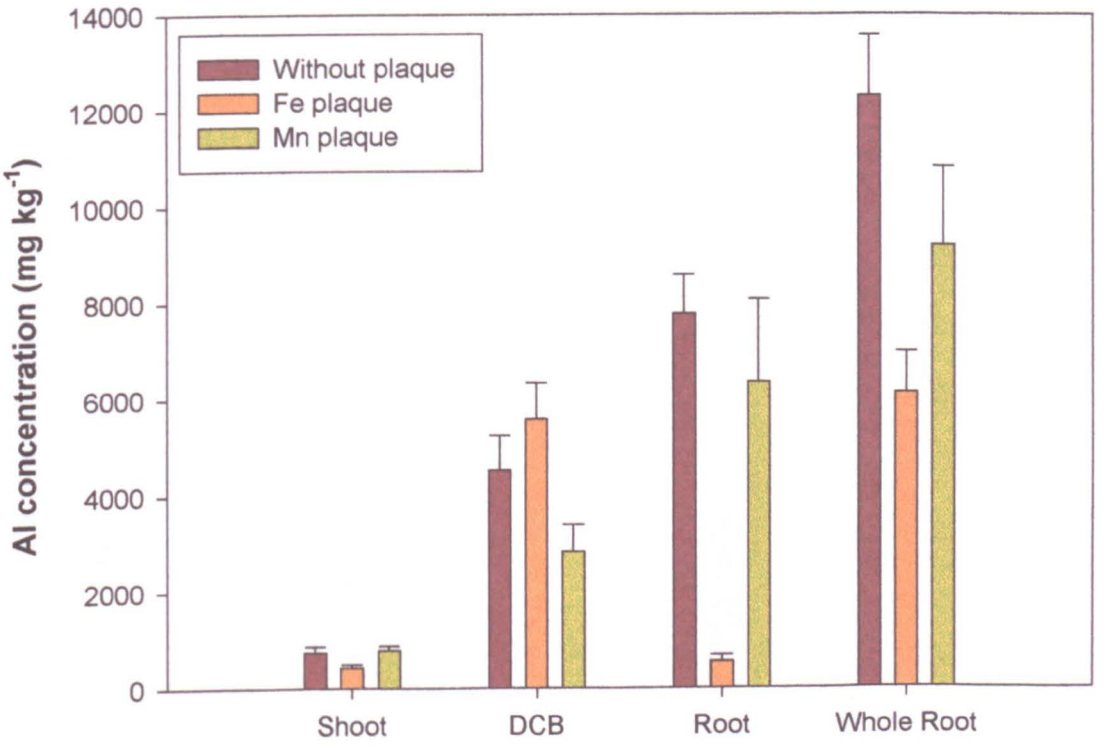


Figure 7.16. The effect of plaque on Al concentration in *P. australis*.

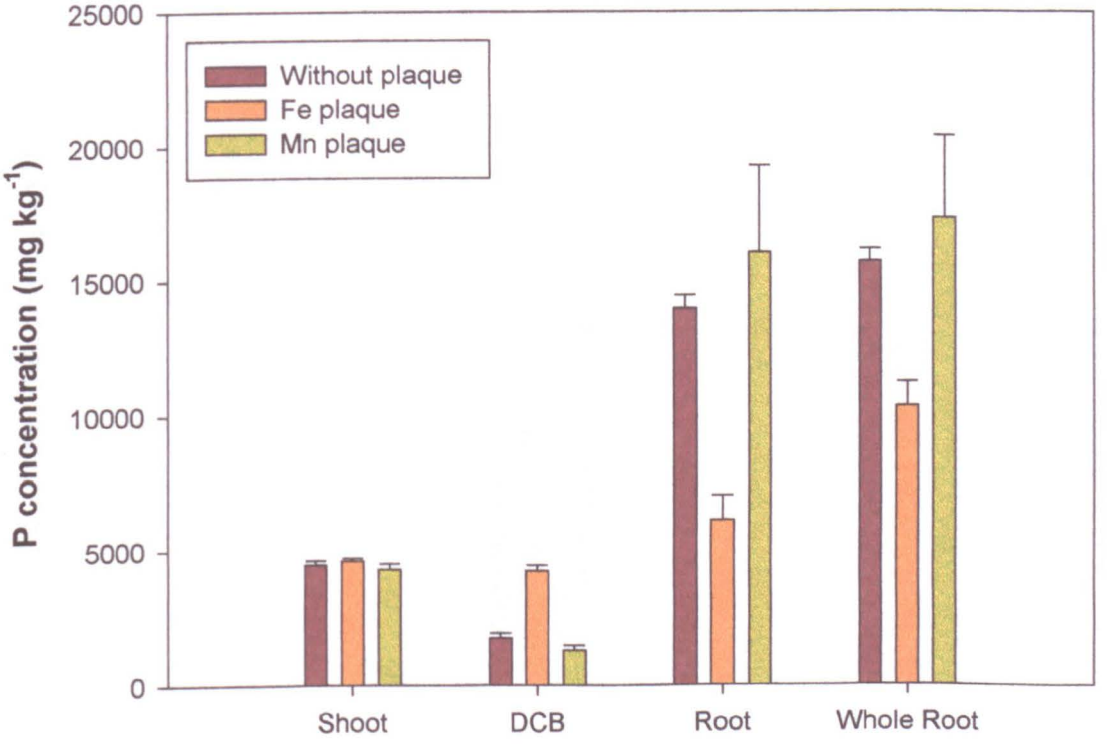


Figure 7.17. The effect of plaque on P concentration in *P. australis* supplied with 1.0 mg l^{-1} Al.

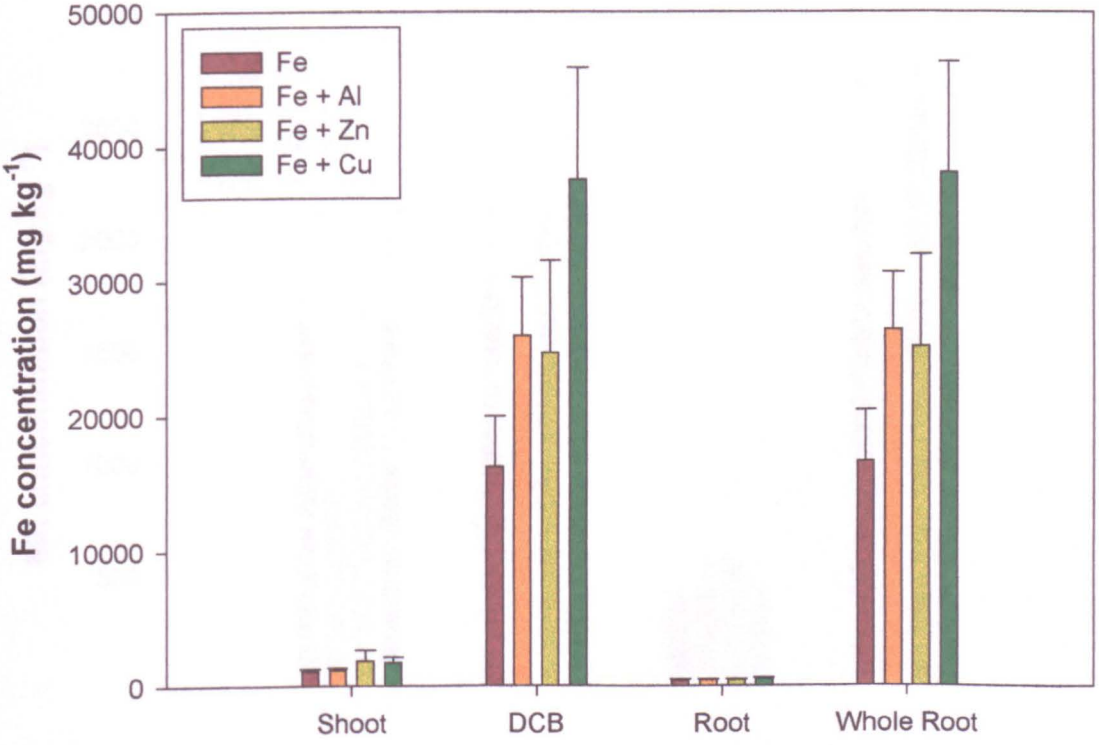


Figure 7.18. The effect of metal supply on Fe concentration in *P. australis*.

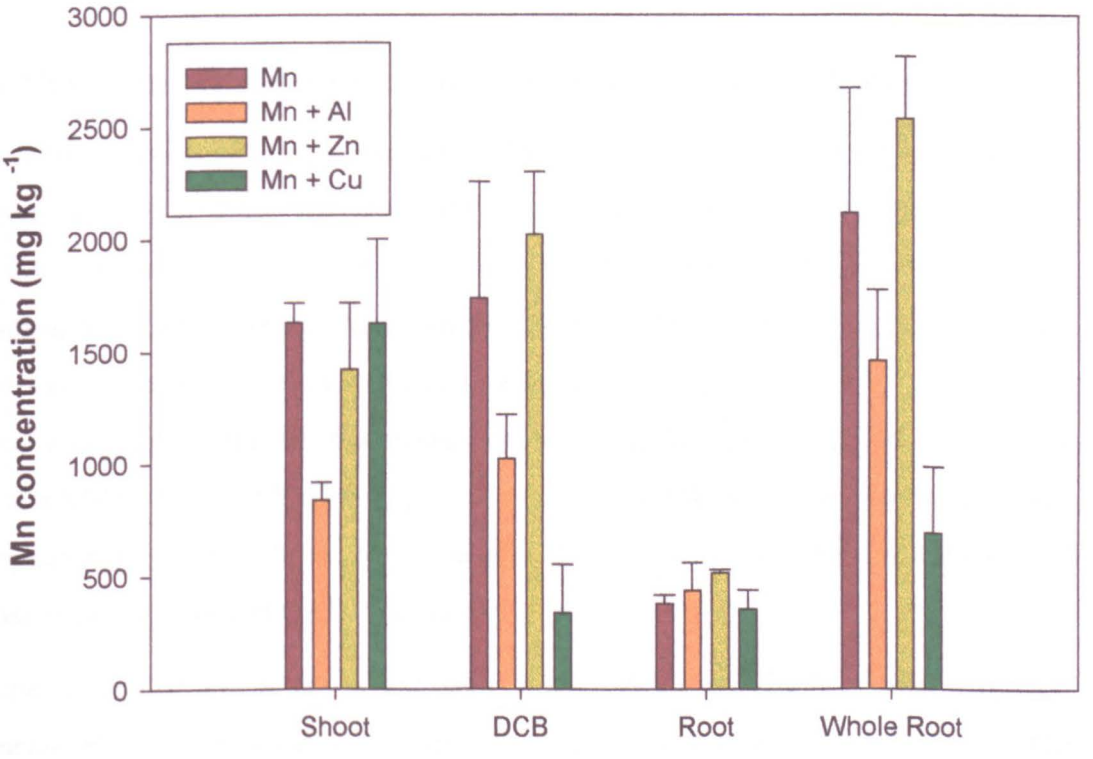


Figure 7.19. The effect of metal supply on Mn concentration in *P. australis*.

7.3.6 The Effect of Accessory Metals on Seedlings Exposed to 50 mg l^{-1} Fe

No significant effect of Al, Cu or Zn was found on the distribution of Fe in *P.australis* seedlings ($p>0.05$). Fe concentration in roots and shoots was very low reaching a maximum of 690.7 and 3432 mg kg^{-1} dry weight, respectively (Figure 7.18). DCB extract values were much higher with values ranging from 11052 mg kg^{-1} to 48281 mg kg^{-1} dry weight.

7.3.7 The Effect of Accessory Metals on Seedlings Exposed to 50 mg l^{-1} Mn

Mn concentration was not significantly different between the different treatments in shoots and roots. However, there was a highly significant effect of accessory metals on Mn concentration in the DCB extract (ANOVA: $F=5.25$, $df=3,8$, $p<0.05$). The concentration was lower in those supplied with Cu than those with Zn or no metals, but was not lower than those with Al to a significant level (Figure 7.19). In addition there was a significant effect of accessory metals on the Mn concentrations in whole roots (ANOVA: $F=4.52$, $df=3,8$, $p<0.05$). Values of Mn were lower in whole roots supplied with Cu than those with Zn added. This was not significantly different from those supplied with either Al or no metals.

There was a much lower concentration of Mn in the DCB extract than the concentrations of Fe in equivalent samples when Fe plaque was present. In the DCB extract Mn concentrations were detected in the range 118-2648 mg kg^{-1} d.w. Mn concentrations were also lower then equivalent Fe concentrations in the roots and shoots with maximum values of 283 and 715 mg kg^{-1} d.w respectively.

7.4 Discussion

The physical characteristics of iron plaques formed in the laboratory have been described in Chapter 6. The Fe plaques produced in the experiment presented here proved to have very similar characteristics, forming an amorphous covering of the root surface which was patchy in its distribution. The plaque also had a definite zonation over the root; it began approximately 1cm from the root tip and darkened with distance from the tip which agrees with previous findings (Taylor *et al.* 1984;

Cook 1990). The plaques were also observed to cover the root hairs, as previously reported (Chapter 6) and there was no evidence of penetration of the plaque into the epidermal cells. Polyhedra and cell casts as reported in Chen *et al.* 1980a were not found in the root samples.

Reports of Mn oxide plaques have been extremely sparse (Bacha & Hossner 1977; Levan & Riha 1986; Crowder & Coltman 1993) and none of these has included investigations into the structure of the plaque using SEM or EDS. Under the naked eye the Mn plaques had a similar distribution to the Fe plaques with formation beginning approximately 1cm from the root tip with a gradual darkening of the plaque with distance from the root tip. Mn plaques differ, however in that they are brown in colour in contrast with the distinctive orange of the Fe plaques. Under the electron microscope the Mn plaques had a similar appearance to Fe plaques. The plaque was composed of an amorphous material which followed the contours of the cells, however the Mn plaque was more patchy in its distribution than the Fe plaque and there were significantly large areas of the root which had no evidence of a plaque deposit. As found for the Fe plaques, there was no evidence of penetration of the Mn plaque into the epidermal cells. This contrasts with the report from Levan & Riha (1986) who found that under a light microscope they could detect the presence of plaque deposits in the cell walls of root epidermis and entire cortex of conifer roots. These examples, however, are non-wetland plants and therefore it is invalid to directly compare these with *P. australis.*

The two types of plaque also differed significantly in their chemistry. Iron plaques that were formed in the laboratory were found to contain P, and was thought to form either a Fe phosphate complex or an iron oxide with P adsorbed to the surface, however this was not clear (Chapter 6). Here, when formed without additional metals, iron plaques were again found to be composed of iron and phosphorus with no accessory metals. However, in some samples of root iron plaque was formed of iron only with no evidence of P. This suggests that the iron does not form an iron phosphate complex, but is present as an iron oxide with P adsorbed to the surface. In some areas adsorption does not occur, although the reason for this is not clear. In contrast Mn plaques were found to be composed of Mn with lesser amounts of Fe and no P. This suggests that Mn does not form a phosphate complex and neither does P

adsorb to the surface of a resultant Mn oxide. The presence of Fe demonstrates that Fe can form plaques on the surfaces of roots even at very low concentrations, in this case at 0.3 mg l^{-1}.

When roots plaqued with iron were supplied with 1.0 mg l^{-1} Al, the plaque had a very different chemistry. Instead of a simple plaque composed mainly of Fe and P, the deposits contained Fe, P and Al. Previously, we have shown that iron plaques are most likely formed of iron oxides with adsorbed P. Fe and Al oxides form readily in the sedimentary environment and so it is likely that Al should also precipitate out around roots in the presence of oxygen as aluminium oxides. These results have important implications in the potential for adsorption of metals onto root plaques, particularly in the presence of P. The sorbing capacity of both iron and aluminium oxides increases with the adsorption of P onto their surfaces (Stanton & Burger 1970; Ghanem & Mikkelson 1988; Kuo & McNeal 1984). The presence of Al was also found on the roots of *P.australis* supplied with Mn, but in this case the plaques were composed of Al and P only and there was no Mn associated with it. This suggests that Mn plaque formation is reduced in the presence of Al, as Al precipitates out more easily around the roots. Al precipitates, composed of Al and P, also formed around the roots of plants without Fe or Mn plaque. These deposits were similar in structure to the Fe and Mn plaques, which formed a patchy, granular coating of the epidermal cells and root hairs. This suggests that aluminium can also form a plaque-type deposit which although not visible with the naked eye, can be identified under the SEM. No previous reports of aluminium plaques have been recorded.

In Chapter 6 we found that neither Cu or Mn were adsorbed onto Fe oxides and concluded that adsorption was not an important mechanism by which plaque prevents the uptake of heavy metals. The results presented here confirm that Cu is not adsorbed onto either Mn or Fe plaques, no Cu was detected by EDS in Mn or Fe plaques when supplied at a concentration of 1.0 mg l^{-1}. However, when supplied with Zn, Mn plaques formed contained trace levels of Zn together with Fe. This suggests that Zn does adsorb onto the surface of plaque to a limited extent. It is possible that Zn is more likely to be adsorbed by Mn oxides than Cu, however in studies of metal adsorption by sediments it was found that Zn is adsorbed to a lesser extent than either Cu or Pb (Hamilton-Taylor *et al.* 1997; Lin & Chen 1998; Machemer & Wildeman

1992). These reports do not address the adsorption characteristics of Mn oxides specifically and therefore the affinity of Mn oxide for Zn could be greater.

The analytical results support some of the findings from the EDS. In the presence of Cu there was no significant effect of plaque on the concentration of Cu in the DCB extract. The EDS results showed that no Cu was adsorbed onto the plaque surface, if adsorption had been an important process then we would have expected more Cu in the DCB extract when plaque was present. However, a significant amount of Cu was found in DCB extracts when plaque is absent which supports the findings from Chapter 6. In the absence of plaque we should get no Cu extracted by the DCB, but the detection of Cu in the extract suggests that it removes metals from within the root and therefore overestimates the amount of metal in plaque. This is also true for when plaque is present.

Lower concentrations of Cu were found in plant shoots when Fe plaque was present and absent than when Mn plaque was present. This was also the case for the plant roots. There was no significant difference between Cu concentrations in all plant parts of those plants with and without Fe plaque. This latter result is in agreement with the findings of Ye *et al.* (1997), who found that there was no significant difference in Cu uptake into the shoots and roots of *T. latifolia* with and without iron plaque. However, in the same report it was shown that Cu on the root surface was higher in the presence of iron plaque. Ye *et al.* (1997) also used the DCB extraction technique and therefore it is possible that the amount of Cu was over-estimated due to the harshness of the extraction technique. A number of other authors have reported that Cu was lower in leaves when plaque was present than absent, but the majority of results were not statistically significant (St Cyr & Crowder 1987; Greipsson & Crowder 1992; Greipsson 1994). Greipsson & Crowder (1992) also reported that Cu accumulated at the root surface whether plaque was present or not, but again, the DCB extraction technique was used and therefore the Cu recorded could have been derived from within the root itself.

The results presented suggest that Fe plaque does not inhibit the uptake of Cu into the plant tissues, however the pH of the system used was pH 3.5 and as previously reported (Chapter 6) the presence of H^+ ions could interfere with the uptake of Cu. This would mask any potential effect of Fe plaque. The formation of Mn plaque,

however, appears to enhance the uptake of Cu into the plant tissues. This was also true for Zn and Al. Otte *et al.* 1989 also found that Fe plaque amplified the uptake of Zn and suggested that this was due to the patchy nature of the plaque. When Fe was supplied at lower concentrations the plaque was more patchy and therefore the Zn was concentrated into a small area by adsorption onto the plaque. They assumed that the root was capable of releasing Zn adsorbed onto the root plaque and therefore would take more up. When plaque is present in higher quantities then the Zn will be more 'dilute' in the plaque and the plant will take less Zn up into tissues. We found that Mn plaque is more patchy than iron plaque and therefore metals could be more concentrated in these precipitates. However, Cu was not found to adsorb onto the surface of plaque and therefore could not have been more concentrated in the small areas of plaque, therefore there must be an alternative explanation for the amplification of Cu uptake in the presence of Mn plaque.

The results from the EDS did show that Zn was adsorbed onto plaque to a certain extent and therefore the theory of Otte *et al.* 1989 could be applied in this example. The uptake of zinc however was only greater for the roots, and as previously shown the results from DCB extract and roots need to be interpreted with care due to the possible extraction of Zn from within the root by the DCB extract. The whole root and shoot data do not reflect this pattern and therefore enhanced uptake is unlikely.

The concentration of Mn in the DCB extract and whole root was less when Mn was supplied in combination with Cu than with Zn or no other metals. This suggests that Cu interferes in some way with the precipitation of Mn oxide. There was also less Mn in the DCB extract and whole roots when Al was supplied. The Al was present as a deposit on the surface of the root which closely resembled an iron plaque, and therefore the Al could be reacting with the available oxygen in preference to the Mn and forming an Al oxide plaque. This would inhibit the formation of Mn plaque, therefore giving lower Mn concentrations in the plaque extract. As a consequence of this, more Mn would be available in the surrounding liquid medium for uptake into the plant tissues, however the Mn concentrations in the shoots were not significantly higher in those plants supplied with Al. This suggests that the aluminium deposit or 'plaque' is also capable of inhibiting the uptake of metals including Mn.

The formation of iron plaques was not affected by the presence of Al which suggests that the Fe is able to compete successfully with Al for reaction with the oxygen available and so iron plaque formation is not inhibited. In addition, the amount of P in the DCB extract is significantly higher when Al is supplied to plants with Fe plaque which is due to the adsorption of P onto the Al and Fe oxides. When Mn plaque is formed P is not adsorbed onto the plaque and therefore P concentrations do not vary. It has previously been suggested that the presence of iron and manganese plaques could prevent the uptake of P through its adsorption onto the plaque surface. Christensen & Sand-Jensen (1998) found that those plants of *Lobelia dortmanna* L. which had a higher concentration of Fe and Mn in plaques also had a lower P value in the aerial parts. However, the detection of P in the plaque was not directly observed and was implied. We have shown that iron does react with phosphate and as a result P accumulates in the iron plaque, but this was not the case for Mn plaque. In addition it was found that the presence of iron or manganese plaques did not lower the level of P in the aerial parts of *P. australis.* The concentration of P in aerial parts was actually higher in plants with Fe and Mn plaques than without, in the controls which is in agreement with previous measurements of P uptake in *Oryza sativa* (Zhang *et al.* 1999). However, it is difficult to directly compare these results as the work of Christensen & Sand-Jensen was carried out on soils obtained from the field as opposed to solution culture in the present case. In addition, as there was no report of the original composition of the soil, other metals may have been present that interfered with the uptake of P.

Concentrations of Zn in the DCB extract reached levels higher than previously reported. Ye *et al.* 1998a found only 963 $\mu g\ g^{-1}$d wt Zn in *T. latifolia* plaques formed in the laboratory despite a greater supply of Zn (2.0 mg l^{-1}). However, the Fe was supplied at a level of only 15 $\mu g\ ml^{-1}$ in contrast with 50 mg l^{-1} in the present study, therefore less plaque would be able to develop and as a result there would be reduced adsorption of Zn onto the plaque surface. Similar concentrations of Zn were found in the shoots indicating that although plaque may be less well developed at the lower concentrations of Fe, it is equally efficient at reducing metal uptake.

No previous work had been carried out on Al uptake in plaqued roots and therefore comparisons cannot be made.

Cu levels in DCB extract were greater than previously reported (Greipsson & Crowder 1994; Ye *et al.* 1997a). This is due to the higher level of Cu supplied in the present study, 1.0 mg l^{-1} as opposed to 0.5 mg l^{-1} in the cited references.

Concentrations of Fe in DCB extracts were similar to previous laboratory experiments (Ye *et al.* 1997, 1998) and field samples (Macfie & Crowder 1989).

Mn concentrations in roots were similar to those found in freshwater sp. from the field (St Cyr & Campbell 1996) but lower than those for *Oryza sativa* grown in the laboratory (Crowder & Coltman 1993) which reached a maximum of 2411 mg kg^{-1} d.w. In the DCB extract similar results were found with a maximum level recorded by Crowder & Coltman (1993) of 10554 mg kg^{-1} d.w. However the experiment using rice was carried out at a range of pH levels and these high concentrations resulted from plaques formed at a pH of 8.0 in contrast with pH 3.5 in the present study. Mn is more likely to precipitate out at this higher pH and therefore it is unsurprising that higher Mn levels result. Values recorded in the same report for plaques formed at pH 3.0 were similar to those found in the present study.

7.5 Conclusions

The formation of iron and manganese plaques has been to shown to occur on the roots of *P. australis* in the laboratory when the elements are supplied at a sufficient level (50 mg l^{-1}). Under the SEM these plaques were similar in appearance, both taking the form of an amorphous coating of the root similar to that seem in Chapter 6. In addition when Al was supplied at a concentration of 1.0 mg l^{-1} this was also found to form plaque deposits which were suggested to be composed of aluminium oxides, These plaques were identical in appearance to Fe plaques, forming over the surface of the cells and around root hairs.

P was found to be associated with both the Fe and Al plaques but not with the Mn plaque deposits. It was suggested that the P was adsorbed to the surface of these plaques rather than forming a compound with them and this could have an important effect on the availability of P to plants.

Fe plaques were found to reduce the uptake of metals in comparison with Mn plaques, and Al plaques were also found to reduce the uptake of Mn although this was not investigated directly. However there was no significant difference in uptake of metals between those plants with and without Fe plaque. This was thought to be due to masking effects of the low pH as seen in Chapter 6. In contrast Mn plaques were shown to amplify the uptake of Zn and Cu but the mechanism by which this is achieved remains unclear.

No metals were found to be adsorbed to the plaque surfaces suggesting that if metal uptake is impeded by the presence of iron plaques then adsorption is not the mechanism by which this occurs. However, Mn oxides were found to adsorb Zn to a certain extent but this does not reduce the uptake of Zn as the chemical analyses indicated an amplification of uptake by the presence of Mn plaques.

These results give rise to a number of areas of research that need to be addressed, in particular the unique report of Al oxide plaques may be important in the growth of plant species in Al-contaminated environments.

8. General discussion

8.1 Introduction

The main aim of the work reported in this thesis was to determine the major metal removal processes in wetlands that receive metal-contaminated drainage waters, and in particular to assess the role of vegetation. The aim of this chapter is not to reiterate the discussions from the previous chapters, but to draw together the major findings and to examine the implications of these with relation to the main objectives of the work. Chapter 1 introduced the chemical and biological characteristics of wetland systems and examined in detail the growth of wetland vegetation. It was suggested that the formation of iron plaques around the roots of plants could constitute a mechanism by which plants growing in contaminated wetlands could avoid the accumulation of phytotoxic metals in their tissues. This has important implications in the use of these plants in constructed wetlands used to remove metals from acid mine drainage. It was also shown that it has been impossible to draw any definitive conclusions from the literature due to inconsistency in research.

The main drawbacks with studies are that they have either been too specific or too general. The use of laboratory based systems allows the investigation of specific processes but does not reflect the natural environment where a number of processes may be involved in producing a particular result. However, the use of field based systems may enable a more accurate representation of natural systems, but they are often too complex to allow the separation of processes. In particular the use of field based systems has been used to investigate the removal of metals in wetland systems but these have tended to concentrate on difference between inputs and outputs and have not investigated the specific processes occurring within the wetland system. In order to address these problems three main approaches were taken in this study.

The first of these utilised field based systems including both natural and constructed wetlands, all of which have received AMD for a number of years allowing complete development of chemical and biological systems including populations of micro-organisms and plant species. In Chapter 4 these were used to determine the processes of metal removal within the wetlands through the use of porewater chemistry. As

expected these systems are extremely complex but it was possible to extract certain patterns of metal distribution and to examine them in conjunction with results from the other chapters. The field based systems were also used (Chapter 6) to examine iron plaques formed in the field. One of the major criticisms of research into plaques carried out in the laboratory is that the plaques may be very different from those formed in the field. It was therefore necessary to establish whether this was the case.

The second approach utilised laboratory based systems using hydroponic cultures to investigate the formation of plaques and their potential ecological role (Chapters 6 and 7). This allowed close control of the chemical environment in which the plants were grown and thus the comparison between different systems.

Finally, a intermediate between these approaches was designed involving the use of mesocosms. This enabled certain control on the system including inputs and outputs, while closely resembling a natural wetland. This was used to determine the main sinks for metals within wetland systems (Chapter 3).

The main conclusions from these experiments are presented below.

8.2 Removal of metals in wetlands.

A number of removal mechanisms have been shown to be significant in wetlands including the presence of vegetation (Sencidiver & Bhumbla 1988; Wildeman & Laudon 1989), formation of iron oxides and sulphides (Feijtel *et al.* 1988; Griffin *et al.* 1989) and adsorption of metals to organic matter (Wieder *et al.* 1990). It was suggested in Chapter 4 that there should be a simple model for the distribution of metals in wetland systems dependant upon the activity of each of the aforementioned processes. However although all the removal mechanisms were thought to occur in the wetlands studied, it was found that distribution of metals did not fit this simple model. This is due to the complexity of natural systems where other factors may also be important in controlling metal transformations. These may include substrate type, AMD composition and supply, type of plant assemblages, flow direction of waters within the wetland and siting of the wetland (i.e. base of slope), all of which need to be considered when considering processes of removal in systems.

The results from Chapters 3, 4 and 5 all suggested that the soil/sediment fraction of the wetland may be the most important sink for metals in wetlands. However, the

type of removal process can vary with location within the system and with time of year. It was indicated that there may be three main removal processes operational in the sedimentary system. In oxidised layers within the wetland the reaction of iron and manganese with oxygen to form metal oxides can remove these contaminants from drainage waters. However, this process is more likely to be important in wetlands receiving less acidic drainage waters, as adsorption of metals onto oxides has been shown to increase with higher pH (e.g. Ghanem & Mikkelson 1988; Fu *et al.* 1991; Coston *et al.* 1995; Jain & Ram 1997). In addition the adsorption of other metals onto these oxides may also potentially remove toxic metals from waters. This is a particularly important process in the upper layers of the wetland profile, but the depth of this layer can vary with season and with the presence of vegetation. At lower depths within the wetland a reducing environment dominates and this allows the reduction of sulphate to sulphide. This sulphide can react with other reduced metals to form metal sulphides including pyrite which can remove contaminants from drainage waters. This may be particularly important for Cu and Zn (Griffin *et al.* 1989), and was suggested in Chapter 4. These minerals tend to be more stable geologically than the oxides and so can provide a more permanent removal process (Luther *et al.* 1986). In addition, sorption of other metals onto the surface of pyrites may also result (Kornicker & Morse 1991). However, the formation of these compounds is controlled by the presence of sufficient quantities of dissolved sulphide, metals and also a supply of organic matter. Thus this removal process may vary in its significance with the type of wetland involved. The third major process in the sedimentary fraction is the adsorption of metals onto organic matter. This may also remove S as compounds are formed by the nucleophilic attack of sulphide onto organic matter (Howarth & Stewart 1992). In Chapter 4 it was shown that overall the levels of metals in profiles were lower in vegetated areas and this could be due to a number of factors including the input of large quantities of organic material, particularly in the autumn. However, adsorption of metals onto sediment particles *per se* is unlikely to be significant in the wetlands studied as the supply of AMD has been continuous over a number of years and so adsorption sites would become saturated as found previously in wetland systems (Machemer & Wildeman 1992). Only by replenishment of oxides and organic matter would adsorption processes continue to be important methods of metal removal.

Metals may also be removed from contaminated drainage by the activity of plants within the wetland. The uptake of metals into plant tissues was shown to occur in the field (Chapter 6) where significant concentrations of a variety of heavy metals were found in the roots and shoots of *P. australis*. *E. angustifolium* and *T. latifolia*. In addition, the formation of iron and manganese plaques was shown to occur in the field thus removing both these metals from the drainage waters. However, these plaques do not adsorb other metals onto their surface and thus do not remove a wide variety of toxic metals and it was also shown in Chapter 3 that there may be release of Fe and Mn back into the environment during the dormant period. This occurred for Fe plaques in *T. latifolia* and for Mn plaques in both *T. latifolia* and *P. australis*.

The formation of iron oxides does not rely on the activity of roots however and may also occur around other organic material including straw (Chapter 3). It was suggested that this iron oxide may differ from that on active roots and may constitute a more stable compound and thus a more stable removal mechanism.

Porewater chemistry was used with some degree of success to examine chemical processes occurring in wetland systems. However, the systems were shown to be highly complex and more information is required on pH and redox amongst other variables within the wetland before the processes can be fully understood using this method. In addition distribution of a number of sulphur species have previously been used to examine metal transformations involving sulphide formation (e.g. Canfield 1989; Smolders & Roelofs 1996). It would be advisable to collect this information from the wetland studied in Chapter 4 to enable a complete assessment of the metal removal mechanisms.

8.3 Characteristics of root plaques.

Direct comparisons were made between plaques formed in the laboratory and those formed in the field. Taylor *et al.* (1984) reported differences between plaques formed in the laboratory and field with plaques less evenly distributed across the root surface in field specimens. However, these field samples of *T. latifolia* were collected in the dormant period and it was shown in Chapter 3 that some dissolution of plaques can

occur in the winter, which would result in this patchy distribution. No significant differences between the physical characteristics of plaques from the field and laboratory were found in Chapter 6. All plaques were found to consist of amorphous particulate coverings which followed the contours of the cells, similar in form to those previously found on roots (Chen *et al.* 1980a; Taylor *et al.* 1984). In addition those plaques formed at low pH were similar to those formed at higher pH with a similar coverage of the root surface. However, plaques formed from manganese rather than iron were less extensive and there were large areas uncoated by plaque deposits as previously reported on the roots of *O. sativa* (Bacha & Hossner 1977). Those plaques formed in the field were also more continuous over the surface of the cells when compared with those from the laboratory. Plaque deposition began approximately 1 cm from the root tip and increased with distance from this region in all cases which is consistent with previous findings (Begg *et al.* 1994). It has been reported that the presence of plaque deposits may inhibit the formation of root hairs (Bacha & Hossner 1977; Taylor *et al.* 1984), but it was shown in Chapters 6 & 7 that root hairs can occur on *P. australis* and that these are often heavily coated with plaque deposits. This has also been shown to occur on roots of *O. sativa* (Otte *et al.* 1989).

The chemical composition of plaques varied greatly and was highly dependent upon the environment in which the plants were grown. Plaques formed in the laboratory and in the field were found to be composed of either Fe, Mn or Al oxides. The formation of Al plaques has not previously been reported and could have important implications for the presence of plant species in Al contaminated sites, although the possibility of Al compound within iron plaques was suggested by Wang & Peverly (1996). Al oxide plaques formed preferentially to Mn plaques, but could occur concurrently with Fe precipitates. It has previously been reported that iron could occur in plaques as iron phosphate (Snowden & Wheeler 1995), however it was shown that iron precipitates could occur without the presence of P and so it is more likely that the P is adsorbed to the surface of both Fe and Al oxide plaques. This could have important significance for the availability of P to plants in natural environments. Templer *et al.* (1998) demonstrated that the presence of *Lythrum salicaria* may indirectly affect the amount of porewater P by having a more oxidised rhizosphere than other plant species studied. Concentrations of P have also been

shown to be higher in plants of *T. latifolia* with an iron plaque than without (Chen *et al.* 1980 a; Crowder *et al.* 1987) indicating that the presence of P on plaques could act as a reservoir when P is limiting.

In Chapter 3 it was suggested that Fe and Mn oxides may adsorb metals including Cu and Zn thereby removing them from solution. This supports previous reports in the literature which have used evidence from DCB extraction techniques to imply the adsorption of metals onto root plaques (Otte *et al.* 1987, 1989; Greipsson & Crowder 1992; Greipsson 1994; Wang & Peverly 1996). However in Chapter 6 and 7 a combination of techniques was used to investigate the possibility of adsorption and co-precipitation. The DCB extraction technique was used as previously reported, but this was used in conjunction with EDS analysis of the root surface. The DCB extracts indicated the presence of metals including Cu within the root plaques, both in Mn and Fe oxides. However EDS analysis showed that there was little evidence of metals associated with the plaques, excepting the presence of Zn with the Mn plaques in Chapter 7. Mn oxides have previously been shown to have a greater affinity for metals at low pH when compared with iron oxides (Fu *et al.* 1991).

It was indicated in Chapter 6 that the DCB extraction could be too harsh as previously suggested in the literature (Bacha & Hossner 1977; McLaughlin *et al.* 1985), and removes metals from within the root tissues thereby giving an overestimation of metal content in the plaques. It is evident therefore that a new extraction technique needs to be developed in order to enable the quantification of metals within root plaques.

8.4 Formation of root plaques.

A number of mechanisms by which plaques form on plant roots have been proposed and were outlined in Chapter 1. It was suggested in Chapter 3 that one of the more important mechanisms could be the release of oxygen from roots through the process of R.O.L. The continued supply of oxygen through the dead culms of *P. australis* by venturi-flow during the dormant period was causally linked to the presence of iron plaques on roots in the winter. In *T. latifolia* where the oxygen release ceases during

dormancy the iron within plaques was remobilised and released back into the surrounding environment. In addition iron oxide plaques were found to be patchy in their distribution in Chapters 6 & 7, which was consistent with the findings of Taylor *et al.* (1984) who reported the correlation of root staining with regions of intense oxygen evolution. However oxygen release was not the only method by which root plaques formed, iron oxides were also found on the surface of straw. These were thought to be a different form of oxide than those formed on active roots as their presence continued through the winter. Microbial action may also be important in the formation of plaques, in particular those formed from Mn and cessation of microbial activity during the winter could result in the remobilisation of these elements. Direct examination of biofilms and micro-organisms however, was outside the scope of this study and requires further consideration. In addition environmental factors including pH and iron availability may also be important in controlling the amount of iron oxide formed (Macfie & Crowder 1987).

Iron plaques were also found to form both at low (3.5) and high (6.0) pH in Chapter 6 which indicates that R.O.L and other plaque-forming mechanisms are not impeded by acidity which could be important to the survival of plant species in acidic environments. However, plaque formation may also be impeded by a rise in pH to a more neutral level. In Chapter 3 it was shown that chemical reduction of previously formed iron plaques on the roots of *T. latifolia* can consume H^+ ions and therefore raise the pH of the rhizospheric environment. This was suggested to lower the availability of Fe for plaque formation on the roots of neighbouring *P. australis* thus inhibiting the ability of these species to form plaque. This would only occur in environments where these two species grow in close proximity to each other, but may be important in mixed species constructed wetlands.

8.5. Ecological role of root plaques.

The presence of plaque on the roots of wetland plants has previously been suggested to constitute a mechanism by which the uptake of potentially phytotoxic metals into plant tissues may be prevented (Marschner 1995; Peverly *et al.* 1995). In Chapter 6 it was shown that Fe plaque reduce the uptake of other metals including Cu into plant tissues. This has been shown to occur in the case of Fe plaques both at low (3.5) and

higher (6.0) pH. Although adsorption and co-precipitation have been proposed as the main process by which metals are prevented from being taken up into plant tissues by the majority of authors (e.g. St Cyr & Crowder 1987), it was not proved in this study. EDS results showed that metals were not adsorbed onto plaque deposits and therefore the plaques must reduce metal uptake by another mechanism.

At low pH it was suggested that the presence of H^+ ions may mask the effects of plaque to some extent, and this was shown to occur in Chapter 7 where the inhibitory effects of Fe and Al plaques were implied but were somewhat disguised by the low pH. The presence of H^+ ions may also constitute an important mechanism by which uptake of metals into plant tissues is impeded. H^+ ions are also released during the formation of iron oxides (Begg *et al.* 1984) and thus the production of iron plaques on roots could produce significant quantities of H^+ ions that inhibit the uptake of other metals. Thus the inhibitory effect of plaques could be due to a by-product of their formation. Alternatively the presence of plaques on the roots could act simply as a physical barrier to the uptake of metals. However, in all cases metal uptake was merely reduced and was not stopped altogether. In samples from the highly contaminated field sites, Parys Mountain and Woolley Colliery, elevated concentrations of a wide range of heavy metals were found in both the root and shoot tissues of plants. Again this suggests that particularly in environments where heavy metal concentration is high, the presence of plaque does not constitute a mechanism of avoiding heavy metal toxicity and there must be alternative mechanisms by which they are able to grow in such stressed conditions.

The adsorption of P onto Fe and Al oxide plaques was reported in Chapter 6 & 7, and the acidity generated during plaque formation may also be significant in increasing the availability of this P for uptake. Begg *et al.* (1984) suggested that the acidity within the rhizosphere could help to mobilise P during periods of restricted availability.

The presence of Mn plaques on the roots of *P. australis* gave a very different result with Cu and Zn uptake into the plants amplified. However the cause of this was unclear. Zn was shown to adsorb onto the Mn oxide plaque and it has been suggested that this is a chemically weaker bond than that formed with sedimentary fractions, therefore the Zn can be made available for uptake. The plaque would thus act as a focus for Zn concentration, thereby allowing greater uptake of Zn upon mobilisation (Otte *et al.* 1989; Zhang *et al.* 1998). It has also been reported that the acidity

generated by Fe oxide formation can increase the availability of Zn for uptake and it may be that this process is also important in Mn oxide formation (Kirk & Bajita 1995). However it is unclear why Cu uptake is also amplified as this element was not adsorbed onto the plaque surface (Chapter 7). The formation of Mn oxides on roots therefore may negatively affect growth of plants which could be important in constructed wetlands receiving high levels of Mn.

Although the adsorption of metals was not found to occur on plaque deposits, it was reported in Chapters 6 and 7 that P could be adsorbed onto the surfaces of Fe and Al plaques. This could have important implications in the availability of P to the plants. However, in low P environments, such as those seen at Parys Mountain, P was not adsorbed onto the plaques and so should be freely available for uptake into plant tissues. If plaque can reduce the uptake of metals by acting as a physical barrier as suggested previously in Chapters 6 & 7 then it is also possible that it could reduce the uptake of nutrients in these low P environments thus having a potentially significant effect on growth.

8.6 Implications for AMD treatment systems.

A variety of species are used in constructed wetlands and there is some debate as to which species is better, and to whether a mixed species system would be more efficient in the removal of heavy metals. In Chapter 3, *P. australis* was shown to be more efficient at removing heavy metals than *T. latifolia* when grown as a monoculture. However, in both cases a monocultural system was more efficient at removing metals from mine drainage than a mixed culture system. *P. australis* was also noted to have a more extensive root system which would allow greater exploitation of the substrate and may also help to reduce the flow rate in wetland systems, thereby allowing greater retention time and thus removal of metals.

However, results from Chapters 4 and 5 suggested that within the whole wetland system sediment-based removal processes may be more important than plant-based processes and as such may be a more important focus for research. The formation of oxides and sulphides may be a significant removal process in many wetlands.

The removal of Mn from contaminated waters has proved to be one of the major difficulties in wetland treatment systems. Mn does not form oxides easily and

chemically does not occur below a pH of 8, which is evidently a problem in wetlands receiving acid drainage. The formation of Mn sulphides may also be impeded as its solubility product is much lower than that of FeS (or CuS and ZnS) so MnS will precipitate only after other metals have been removed (Wieder 1993). In addition it has been shown that sulphidation of Mn oxides results not in the precipitation of Mn sulphide but instead the oxidation of sulphide to sulphur or sulphate with release of Mn into solution (Tarutis & Unz 1995). Therefore although sulphide formation may be important in wetlands for removal of Cu, Zn and Fe, this is not the case for Mn as shown at Parys Mountain. In addition it has been shown that the adsorption of Mn onto organic matter is not an important removal mechanism.

It is possible that plants in constructed and natural wetlands may not be important in the removal of metals directly but contribute significantly to other processes. The production of sulphide minerals is dependent upon a continuing supply of organic matter and thus the presence of macrophytes, particularly those with a high litter production could facilitate this process. In addition the presence of plants in wetlands systems can help to increase the retention time of contaminated waters, allowing for greater potential for reactions to occur, and can aid in the trapping of contaminated sediment particles (Wildeman & Laudon 1989). They may also act as a focus for microbial action which may be important in the production of oxides and sulphides within wetland systems.

8.7 Conclusions

It is evident that wetland systems are extremely complex and a large variety of processes may be active concurrently and at different times in the year which act to remove metals from AMD. However a number of main conclusions can be obtained from this study.

Although vegetation has not been shown to constitute an important removal mechanism itself through the uptake of metals, it may be significant in affecting a number of chemical processes. It has been suggested that the presence of vegetation within wetland systems can remove metals by the following processes

1. Filtering suspended and colloidal material from water
2. Adsorption/exchange of contaminants onto soil material, live plant material and dead plant material
3. Precipitation and neutralisation through generation of NH_3 and HCO_3^- by bacterial decay of biological material. (Wildeman & Laudon 1989).

The mechanism by which plant species survive in these highly contaminated environments however remains unclear. Plaque formation has been proposed as one mechanism by which plants prevent the uptake of phytotoxic levels of metals into tissues. In the study presented here it was shown that iron plaques form on a number of wetland species and may be composed of iron, manganese or aluminium oxides dependent upon the chemical environment. The presence of Fe and Al plaques reduced the uptake of other metals including Cu and Zn but did not prevent it and Mn plaques accelerated the uptake of these metals. The method by which uptake is reduced has previously been thought to be the adsorption onto and co-precipitation of metals with the plaque deposits, however EDS analysis indicated that this was not the case. Instead the inhibition of uptake was suggested to be caused by the presence of H^+ ions or to the plaque acting as a physical barrier. However, in highly contaminated environments uptake of metals is not significantly reduced and thus there must be another mechanism active by which plants are able to survive.

Overall within wetland systems, the formation of oxide and sulphide minerals may be a more important sink for heavy metals than any of the other sinks. However, in particular the formation of stable sulphide minerals is dependent upon a continuous supply of organic matter and thus the presence of macrophytes may be important in this role.

It is clear that there are a number of different mechanisms active within wetlands that act to remove metals but the presence of macrophytes can positively contribute to the activity of these removal processes. The addition of vegetation to wetland treatment systems is therefore essential if metal removal is to be achieved.

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Appendix A.

Chemical composition of Rorison's solution

Element	Concentration ($mg\ l^{-1}$) in full strength solution	Source compound	Stock solutions		Volume of stock required for 1l nutrient solution (ml)	
			1L	500ml	Full strength	1/10 strength
Ca **N**	80 56	$Ca(NO_3)_2.4H_2O$	94.27g	47.14g	5	0.5
Mg	24	$MgSO_4.7H_2O$	243.32g	121.66g	1	0.1
K **P**	78 31	K_2HPO_4 (trihydrate)	173.74g (227.65g)	86.87g (113.82g)	1	0.1
Fe	3.0	FeNaEDTA	19.72g	9.86g	1	0.1
Mn	0.5	$MnSO_4.4H_2O$	2.0301g	1.0151g	1	0.1
B	0.5	H_3BO_3	2.8596g	1.4298g		
Mo	0.1	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.1840g	0.0920g		
Zn	0.1	$ZnSO_4.7H_2O$	0.4401g	0.2200g		
Cu	0.1	$CuSO_4.5H_2O$	0.3298g	0.1964g		

Appendix B.

Reagent 1 (ammonium molybdate solution) used in the analytical procedure for phosphorus.

Dissolve 5g of Ammonium molybdate, $(NH_4)_6MO_7O_{24}.\ 4H_2O$) in 300 ml UHP water. Add 17.5 ml concentrated sulphuric acid slowly while stirring and dilute to 500 ml. De-gas. The reagent is stable for several months.

Appendix C.

Reagent 2 (stannous chloride) used in the analytical procedure for phosphorus.

Add 14 ml of concentrated sulphuric acid slowly to 300 ml of UHP water while stirring. Dissolve 0.1 g Stannous chloride ($SnCl_2.\ 2H_2O$) and 1g Hydrazinium sulphate ($N_2H_6SO_4$) in the acid solution and dilute to 500 ml. De-gas. The reagent is stable for several weeks.

Appendix D.

Results of statistical analyses from Chapter 3.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot†	<0.001	14.7	3,16	NS	0.2	1,16	NS	0.7	3,16
DCB	NS	3.4	2,12	<0.05	5.4	1,12	<0.05	5.7	2,12
root†	<0.001	14.1	2,12	<0.001	239.8	1,12	<0.05	6.1	2,12
whole root	NS	2.0	2,12	NS	4.2	1,12	<0.01	9.9	2,12
soil	<0.01	4.6	4,20	<0.001	28.7	1,20	<0.001	13.1	4,20
pore-water	<0.001	7.0	4,20	NS	3.8	1,20	<0.05	2.9	4,20

Statistical analysis of Fe concentration in single species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot	<0.001	23.3	3,16	<0.05	5.9	1,16	NS	1.1	3,16
DCB	NS	0.2	2,12	<0.01	12.7	1,12	NS	0.2	2,12
root†	<0.05	6.3	2,12	NS	2.3	1,12	<0.01	7.5	2,12
whole root†	NS	0.04	2,12	<0.05	6.6	1,12	NS	0.1	2,12
soil	NS	0.6	4,20	<0.01	13.3	1,20	<0.001	7.4	4,20
pore-water†	<0.001	12.2	4,20	<0.001	32.2	1,20	<0.05	2.9	4,20

Statistical analysis of Mn concentration in single species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot†	<0.001	28.0	3,16	<0.05	4.7	1,16	NS	0.2	3,16
DCB†	NS	0.1	2,12	<0.001	47.0	1,12	NS	1.4	2,12
root	<0.001	23.6	2,12	<0.001	41.2	1,12	<0.05	6.0	2,12
whole root	NS	3.5	2,12	NS	4.1	1,12	NS	2.3	2,12
soil	NS	1.0	4,20	NS	0.4	1,20	NS	1.9	4,20
pore-water									

Statistical analysis of Al concentration in single species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot	<0.001	13.4	3,16	NS	0.5	1,16	NS	0.5	3,16
DCB†	<0.05	5.0	2,12	<0.001	134.0	1,12	<0.05	4.4	2,12
root†	<0.001	45.9	2,12	<0.001	236.2	1,12	<0.001	14.5	2,12
whole root†	<0.01	11.4	2,12	<0.001	175.2	1,12	<0.001	15.0	2,12
soil	<0.01	6.3	4,20	<0.05	5.4	1,20	NS	1.6	4,20
pore-water	NS	2.1	4,20	NS	0.9	1,20	NS	1.4	4,20

Statistical analysis of Zn concentration in single species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot	<0.001	12.2	3,16	<0.05	5.2	1,16	NS	1.7	3,16
DCB†	NS	1.0	2,12	NS	1.5	1,12	NS	0.1	2,12
root†	NS	1.5	2,12	<0.05	6.7	1,12	NS	0	2,12
whole root	NS	0.3	2,12	<0.05	4.8	1,12	NS	1.2	2,12
soil	<0.05	3.0	4,20	<0.001	16.2	1,20	NS	1.2	4,20
pore-water†	NS	2.1	4,20	NS	0.4	1,20	NS	0.8	4,20

Statistical analysis of Cu concentration in single species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot	<0.001	14.4	3,16	<0.05	7.9	1,16	<0.05	3.6	3,16
DCB	NS	0.02	2,12	NS	0.7	1,12	NS	3.2	2,12
root†	<0.05	5.1	2,12	NS	0.5	1,12	NS	0.4	2,12
whole root	NS	3.3	2,12	<0.05	5.5	1,12	<0.01	8.2	2,12
soil	<0.01	5.7	4,20	NS	1.0	1,20	<0.001	10.8	4,20
pore-water	NA	NA	NA	NA	NA	NA	NA	NA	NA

Statistical analysis of P concentration in single species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot	NS	0.1	2,11	NS	3.2	1,11	NS	0.4	2,11
DCB†	NS	1.9	2,11	NS	0.4	1,11	NS	0.1	2,11
root†	<0.001	13.0	2,11	<0.001	87.5	1,11	<0.01	8.0	2,11
whole root	NS	3.8	2,11	NS	2.6	1,11	NS	1.2	2,11

Statistical analysis of Fe concentration in mixed species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot	<0.001	15.0	2,11	NS	4.2	1,11	NS	2.4	2,11
DCB	NS	1.3	2,11	<0.05	6.8	1,11	NS	0.4	2,11
root†	<0.01	11.3	2,11	<0.05	5.8	1,11	<0.001	13.3	2,11
whole root	NS	3.3	2,11	NS	2.4	1,11	NS	0.1	2,11

Statistical analysis of Mn concentration in mixed species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot	NS	0.7	2,11	NS	0.1	1,11	NS	0.3	2,11
DCB	NS	2.8	2,11	<0.001	35.7	1,11	NS	2.9	2,11
root	<0.05	4.8	2,11	<0.05	8.1	1,11	NS	2.7	2,11
whole root	<0.05	6.6	2,11	<0.05	7.2	1,11	NS	0.3	2,11

Statistical analysis of Al concentration in mixed species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot	<0.05	4.7	2,11	NS	0	1,11	NS	0.2	2,11
DCB	NS	1.2	2,11	<0.05	10.3	1,11	NS	0.6	2,11
root	<0.001	26.0	2,11	<0.001	131.4	1,11	<0.001	24.5	2,11
whole root†	NS	0.3	2,11	<0.001	20.6	1,11	NS	0.1	2,11

Statistical analysis of Zn concentration in mixed species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot †	<0.01	8.2	2,11	NS	0.1	1,11	NS	2.0	2,11
DCB	NS	1.2	2,11	NS	2.4	1,11	NS	0.9	2,11
root	<0.001	17.6	2,11	<0.001	5.61	1,11	<0.001	19.1	2,11
whole root	NS	2.2	4,11	NS	1.4	1,11	NS	1.4	4,11

Statistical analysis of Cu concentration in mixed species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Vegetation			Season			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
shoot†	<0.05	4.4	2,11	<0.01	17.4	1,11	NS	0.4	2,11
DCB	NS	1.6	2,11	NS	0.3	1,11	NS	0.2	2,11
root†	NS	3.4	2,11	NS	0.2	1,11	<0.001	12.4	2,11
whole root†	<0.05	4.2	2,11	NS	0.1	1,11	<0.05	6.1	2,11

Statistical analysis of P concentration in mixed species cultures. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

Appendix E

Results of statistical analyses from Chapter 4.

Parys Mountain

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter†	<0.001	13.7	4,77	NS	0.6	9,77	NS	1.1	36,77
Spring†	<0.001	21.0	3,68	NS	0.6	9,68	NS	0.6	27,68
Summer†	<0.001	30.0	4,99	NS	2.0	9,99	NS	0.8	36,99
Autumn†	<0.001	38.3	3,78	NS	1.2	9,78	<0.05	1.7	27,78

Statistical analysis of Fe concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	<0.001	156.1	4,77	NS	0.3	9,77	NS	0.6	36,77
Spring	<0.001	92.8	3,72	<0.001	4.6	9,72	<0.001	2.9	27,72
Summer	<0.001	367.6	4,98	<0.001	5.6	9,98	<0.05	1.7	36,98
Autumn†	<0.001	92.6	3,78	<0.01	2.7	9,78	<0.001	2.6	27,78

Statistical analysis of Mn concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	<0.001	88.5	4,77	<0.01	3.0	9,77	<0.001	3.9	36,77
Spring†	<0.001	48.1	3,73	NS	0.6	9,73	<0.001	2.7	27,73
Summer†	<0.001	200.4	4,97	<0.001	7.2	9,97	<0.001	8.5	36,97
Autumn†	<0.001	6.3	3,78	<0.001	8.2	9,78	<0.001	3.6	27,78

Statistical analysis of Al concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	<0.001	43.5	4,77	<0.05	2.6	9,77	NS	0.8	36,77
Spring†	<0.001	26.1	3,72	NS	0.8	9,72	<0.05	1.9	27,72
Summer†	<0.001	70.2	4,95	<0.001	5.2	9,95	<0.001	2.3	36,95
Autumn†	<0.01	4.2	3,78	<0.001	5.7	9,78	NS	1.0	27,78

Statistical analysis of Zn concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter†	<0.001	27.8	4,73	<0.01	3.3	9,73	NS	1.2	36,73
Spring†	<0.05	3.7	3,66	NS	0.7	9,66	NS	1.5	27,66
Summer†	<0.001	16.1	4,98	<0.001	4.2	9,98	<0.001	3.0	36,98
Autumn†	<0.001	62.5	3,78	NS	1.0	9,78	NS	1.5	27,78

Statistical analysis of Cu concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter†	<0.001	19.8	4,74	<0.001	3.8	9,74	NS	0.8	36,74
Spring									
Summer†	<0.001	21.7	4,97	<0.01	2.8	9,97	<0.001	3.8	36,97
Autumn†	<0.001	32.4	3,78	<0.001	10.7	9,78	<0.05	1.8	27,78

Statistical analysis of Pb concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	<0.001	46.0	4,76	NS	1.6	9,76	NS	1.0	36,76
Spring†	<0.001	11.9	3,72	NS	1.8	9,72	NS	1.2	27,72
Summer	<0.001	286.7	4,92	<0.05	2.3	9,72	NS	1.5	36,92
Autumn†	<0.001	76.7	3,78	<0.01	2.7	9,78	NS	1.4	27,78

Statistical analysis of Ca concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	<0.001	89.7	4,76	NS	0.4	9,76	NS	0.6	36,76
Spring	<0.001	165.0	3,71	<0.001	11.4	9,71	<0.001	7.5	27,71
Summer	<0.001	269.0	4,98	<0.001	6.8	9,98	NS	1.4	36,98
Autumn†	<0.001	53.4	3,78	<0.05	2.2	9,78	NS	1.3	27,78

Statistical analysis of Mg concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter†	<0.001	45.1	4,77	NS	1.0	9,77	NS	1.0	36,77
Spring	<0.001	103.6	3,72	<0.001	9.5	9,72	<0.001	5.0	27,72
Summer†	<0.001	135.5	4,97	<0.001	4.9	9,97	NS	1.5	36,92
Autumn	<0.001	4.6	3,78	<0.001	4.7	9,78	NS	1.2	27,78

Statistical analysis of S concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter									
Spring									
Summer†	<0.001	22.1	4,97	NS	1.2	9,97	<0.001	3.4	36,97
Autumn†	NS	1.7	3,78	<0.001	9.4	9,78	NS	1.1	27,78

Statistical analysis of P concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

Mam Tor

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter†	NS	0.8	3,48	NS	0.4	6,48	NS	0.6	18,48
Spring†	<0.001	25.1	2,56	<0.001	13.5	9,56	<0.01	2.7	18,56
Summer†	NS	2.6	3,70	NS	1.6	9,70	<0.01	2.2	27,70
Autumn†	<0.001	12.6	3,54	<0.001	5.4	6,54	<0.001	4.7	18,54

Statistical analysis of Fe concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	<0.001	8.4	3,51	NS	1.2	6,51	NS	0.9	18,51
Spring	<0.05	3.7	2,56	<0.001	11.0	9,56	NS	1.0	18,56
Summer	<0.001	23.4	3,70	<0.001	6.6	9,70	<0.001	2.9	27,70
Autumn†	<0.001	32.4	3,54	<0.001	5.2	6,54	<0.01	2.6	18,54

Statistical analysis of Mn concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	NS	2.5	3,51	NS	1.4	6,51	NS	0.5	6,51
Spring†	<0.001	12.7	2,47	<0.001	5.8	9,47	<0.05	2.0	18,47
Summer†	<0.001	37.1	3,71	NS	0.4	9,71	<0.001	4.5	27,71
Autumn†	<0.001	27.0	3,49	NS	2.3	6,49	<0.001	4.0	18,49

Statistical analysis of Al concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter†	<0.001	7.8	3,51	NS	0.4	6,51	NS	1.2	18,51
Spring	NS	0.4	2,48	<0.001	5.5	9,48	NS	1.3	18,48
Summer†	<0.001	33.0	3,69	NS	1.8	9,69	<0.001	3.8	27,69
Autumn†	<0.001	15.5	3,48	NS	1.3	6,48	NS	1.3	18,48

Statistical analysis of Zn concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter									
Spring†	<0.001	11.9	2,57	<0.001	6.1	9,57	NS	1.1	18,57
Summer†	<0.001	15.9	3,70	<0.001	8.9	9,70	<0.01	2.1	27,70
Autumn†	<0.001	14.2	3,54	<0.001	4.9	6,54	NS	1.0	18,54

Statistical analysis of Cu concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter									
Spring†	NS	2.1	2,57	<0.05	2.3	9,57	NS	0.7	18,57
Summer†	<0.001	20.0	3,70	<0.001	8.3	9,70	<0.001	2.9	27,70
Autumn†	<0.001	10.8	3,55	NS	1.4	6,55	NS	1.2	18,55

Statistical analysis of Pb concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	<0.001	21.5	3,50	NS	1.2	6,50	NS	1.0	18,50
Spring†	<0.001	9.7	2,44	<0.001	7.1	9,44	NS	1.1	18,44
Summer	<0.001	11.2	3,68	<0.001	6.1	9,68	<0.001	2.9	27,68
Autumn	<0.001	16.6	3,54	<0.001	6.4	6,54	NS	1.4	18,54

Statistical analysis of Ca concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter	NS	2.1	3,50	NS	0.3	6,50	NS	0.2	18,50
Spring	<0.01	6.6	2,47	<0.001	8.4	9,47	NS	0.6	18,47
Summer	<0.01	5.1	3,71	<0.01	3.3	9,71	NS	1.6	27,71
Autumn	<0.001	8.8	3,51	<0.01	3.8	6,51	NS	1.5	18,51

Statistical analysis of Mg concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter‡	<0.05	3.3	3,50	NS	0.9	6,50	NS	0.9	18,50
Spring†	<0.01	7.4	2,47	<0.001	6.6	9,47	NS	1.7	18,47
Summer†	<0.001	6.6	3,71	<0.001	5.7	9,71	<0.001	4	27,71
Autumn†	<0.001	20.0	3,52	NS	1.2	6,52	<0.01	2.4	18,52

Statistical analysis of S concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (GLM)								
	Site			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Winter									
Spring†	NS	2.5	2,57	<0.05	2.1	9,57	NS	0.4	18,57
Summer†	<0.001	18.9	3,70	<0.001	18.6	9,70	<0.001	3.7	27,70
Autumn†	<0.05	3.0	3,54	NS	0.6	6,54	NS	1.3	18,54

Statistical analysis of P concentration in porewaters. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

Appendix F

Results of statistical analyses from Chapter 5.

	Factorial analysis (GLM)								
	Vegetation			Depth			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Fe	<0.001	24.5	3,104	<0.01	2.7	9,104	NS	0.4	27,104
Mn†	<0.001	21.0	3,104	<0.001	4.5	9,104	NS	1.4	27,104
Al†	<0.001	20.4	3,104	<0.001	17.5	9,104	NS	1.4	27,104
Zn†	<0.001	25.0	3,104	<0.001	18.9	9,104	NS	0.6	27,104
Cu†	<0.001	12.1	3,104	<0.001	9.7	9,104	NS	1.0	27,104
Pb†	<0.001	15.0	3,104	<0.001	5.9	9,104	NS	0.6	27,104
Ca†	<0.001	266.6	3,104	<0.001	17.3	9,104	<0.001	2.7	27,104
Mg†	<0.001	289.3	3,104	<0.001	15.1	9,104	<0.001	3.8	27,104
K†	<0.001	40.8	3,104	<0.001	3.5	9,104	NS	1.2	27,104
Na†	<0.001	8.9	3,104	NS	0.9	9,104	NS	0.6	27,104
S‡	<0.001	583.1	3,104	<0.001	12.1	9,104	<0.001	2.6	27,104
P†	<0.001	12.0	3,104	<0.001	8.7	9,104	<0.05	1.8	27,104

Statistical analysis of metal concentrations in porewaters. †denotes $\log_e$ transformed data, ‡ denotes $\log_{10}$ transformed data.

Appendix G

Results of statistical analyses from Chapter 6.

	Factorial analysis (Two-way ANOVA)								
	Plaque			pH			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Shoot	NS	0.04	1,8	NS	1.6	1,8	NS	5.0	1,8
DCB‡	<0.05	5.8	1,8	<0.001	20.7	1,8	NS	2.1	1,8
Root†	NS	0.3	1,8	NS	0.3	1,8	NS	4.4	1,8
Whole Root†	<0.05	10.0	1,8	<0.001	26.9	1,8	NS	3.3	1,8

Statistical analysis of Mn concentration in *P. australis* grown in solution culture. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (Two-way ANOVA)								
	Plaque			pH			Interaction		
	P	F	d,f	P	F	d,f	P	F	d,f
Shoot†	NS	4.6	1,8	<0.05	9.6	1,8	<0.05	7.6	1,8
DCB†	NS	1.6	1,8	<0.05	7.4	1,8	NS	0.4	1,8
Root‡	<0.001	45.4	1,8	<0.001	27.4	1,8	<0.05	9.7	1,8
Whole Root	<0.05	6.8	1,8	<0.01	14.2	1,8	NS	4.9	1,8

Statistical analysis of Cu concentration in *P. australis* grown in solution culture. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

Appendix H

Results of statistical analyses from Chapter 7.

	Factorial analysis (One-way ANOVA)								
	Copper			Aluminium			Zinc		
	P	F	d,f	P	F	d,f	P	F	d,f
Shoot	†<0.01	11.2	2,6	†<0.05	6.08	2,6	<0.01	12.0	2,6
DCB	NS	2.0	2,6	<0.001	4.1	2,6	NS	4.6	2,5
Root	<0.05	5.7	2,6	†<0.001	33.6	2,6	†<0.01	11.8	2,6
Whole Root	‡NS	2.2	2,6	<0.05	5.8	2,6	‡NS	0.6	2,6

Statistical analysies of the effect of plaque treatment on metal concentration. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

	Factorial analysis (One-way ANOVA)											
	Copper			Aluminium			Zinc			No metals		
	P	F	d,f	P	F	d,f	P	F	d,f	P	F	d,f
Shoot	†NS	0.8	2,6	NS	0.9	2,6	†NS	1.6	2,6	‡<0.05	8.0	2,6
DCB	‡<0.05	12.5	2,4	<0.001	68.4	2,6	†NS	2.6	2,6	†<0.01	16.0	2,6
Root	NS	3.4	2,6	†<0.05	14.0	2,6	†NS	0.7	2,6	‡NS	1.6	2,6
Whole root	‡NS	2.4	2,6	‡<0.05	6.0	2,6	†NS	0.2	2,6	†NS	1.2	2,6

Statistical analysies of the effect of plaque treatment on P concentration. Analyses used untransformed data except for † $\log_e$ transformed, and ‡ $\log_{10}$ transformed.

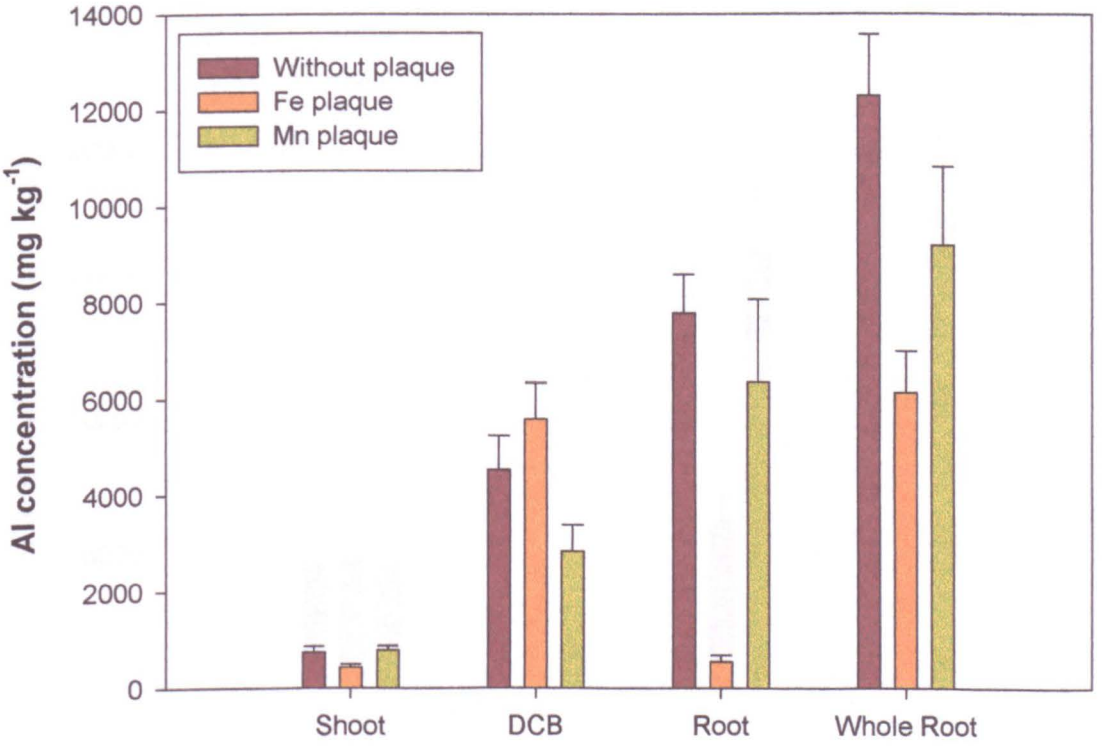

Figure 7.16. The effect of plaque on Al concentration in *P. australis*.

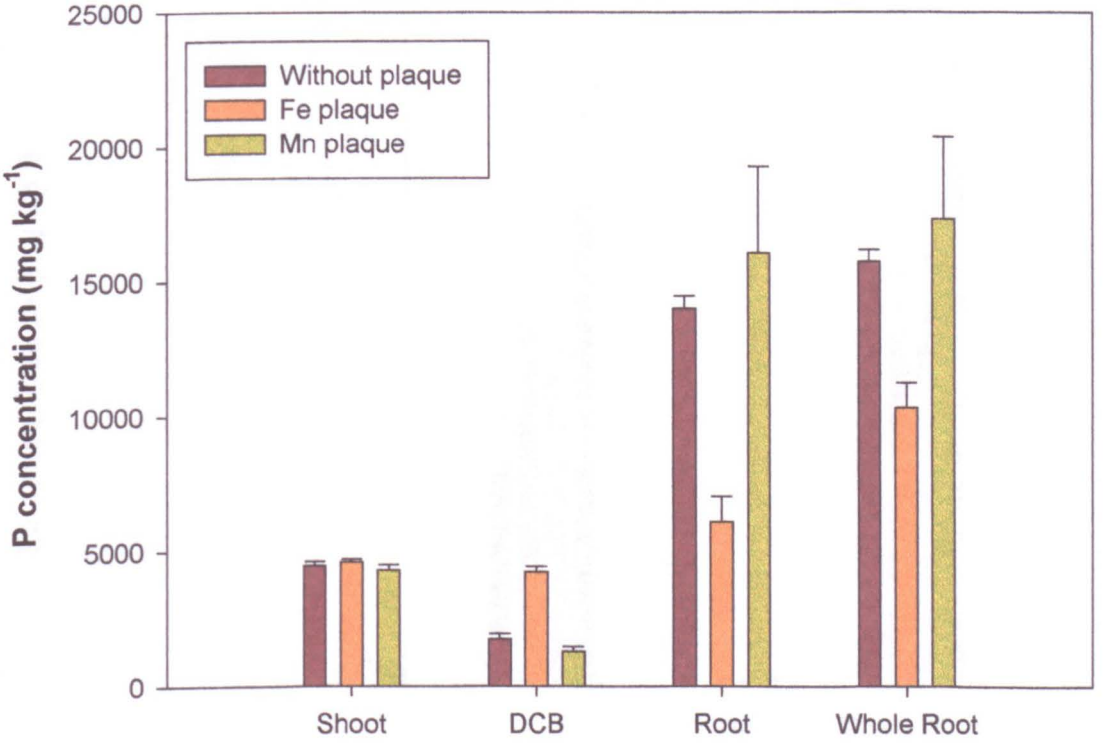

Figure 7.17. The effect of plaque on P concentration in *P. australis* supplied with 1.0 mg l^{-1} Al.

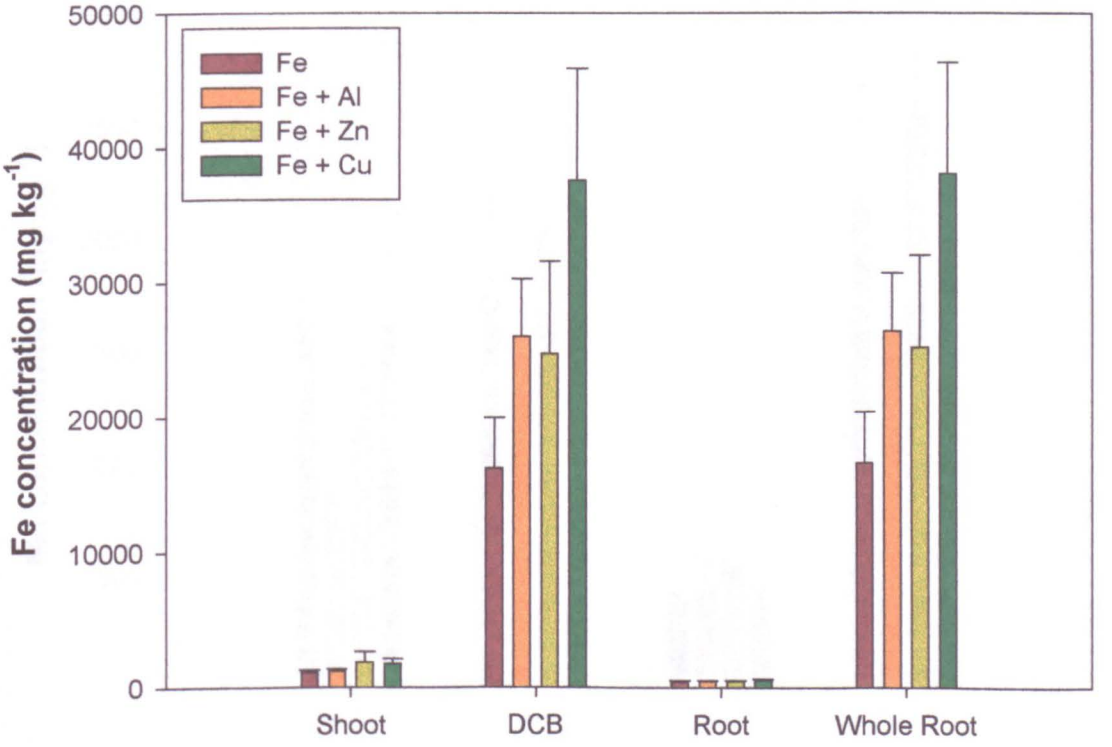

Figure 7.18. The effect of metal supply on Fe concentration in *P. australis*.