Characterisation and Remediation of a Canal Sediment Contaminated with Heavy Metals and Organic Pollutants

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This thesis is submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

Collaborating Institutions: The University of Liverpool

October 2005

ACKNOWLEDGEMENTS

Firstly I would like to acknowledge the help of my supervisors Professor N. M. Dickinson and Dr P. Putwain both for offering me the opportunity to undertake this PhD and also for their help during the past 3 years. Thanks to Nick for his rapid reading and correcting of chapters.

A massive thank you also to the staff at CSIRO Land & Water in Australia, especially Mike McLaughlin, Annette Nolan (and Brett), Enzo Lombi, Tündi Heinrich, Rebecca Hamon and Daryl Stevens for not only the fantastic opportunity which they gave me, but also their help and friendship throughout my time there. Without them, Chapters 4 and 6 would not have been possible.

Dr Ian Pulford has been wonderful and I would like to thank him very sincerely for his help and his suggestions. Thank you to Mike O'Connor for help with the fieldwork and provision of photographs. Thank you also to the various technical staff (including Mark Blackburn and Vicki Mills) for their help with this project.

On a more personal note I'd like to thank Roz King for not only being a good friend and an insufferable optimist, but also for all of the advantages that her powerful personality brought to the project. She has been a joy to work with and has made this PhD a truly unforgettable experience.

I would also like to thank Tony for his unwavering support and for the proof reading of so many pages of unfamiliar science.

Without the support and funding provided by Warrington Borough Council, Peel Holdings Ltd., Littlewoods, and the Northwest Development Agency none of this work would have been possible and the Woolston New Cut Canal would have retained its secrets to this day.

Finally, I would like to dedicate this thesis to me, for all my hard work.

ABSTRACT

Sediment was dredged from a disused canal site and planted with *Salix*, *Populus* and *Alnus* species for the purpose of heavy metal and organic pollutant phytoremediation. Heavy metal behaviour during sediment oxidation was studied in laboratory and pot trials using an anoxic sequential extraction scheme, isotopic dilution techniques, diffusive gradients in thin films (DGT) and a root elongation study. A pot trial investigated the effects of environmental variables on tree biomass production. The high organic matter content and the lack of pH buffering carbonate minerals in this sediment made it a novel substrate for study. A drop in pH from ~ 6 to ~ 3.5 occurred during oxidation of the sediment which, coupled with the increasing redox potential, led to increased heavy metal mobility, lability and phytotoxicity. The acidification led to an increase in Zn mobility of 1670 % over a 48-day oxidation period in a pot trial. Addition of ameliorants increased the pH of the oxidised sediment and decreased mobility and pore water concentrations of heavy metals and also reduced heavy metal flux from labile solid phases into solution. Consequently there was an increase in biomass production in concorde ryegrass growing in lime-amended sediment when compared to unamended sediment, and a decrease in heavy metal phytoextraction. Only the lime-containing amendments proved to be effective in ameliorating this sediment due to its low pH. The use of DGT techniques yielded a good relationship between the effective concentration (C_E) and the concentration of heavy metals in plant tissue. In the pot trial designed to assess the effect of environmental variables on tree biomass production, biomass yield was greatest when the trees were growing in deeper, wetter sediment, and the lowest biomass production occurred when the trees were growing in shallower, drier sediment. Heavy metal mobility and phytoaccumulation were decreased in the former treatment with respect to the latter. Biomass production at the canal site appeared to follow these same trends. Generally, biomass production at the canal site was low and phytoextraction was not as high as has been reported in the literature. *Salix* X Calodendron showed the greatest heavy metal phytoremediation potential (higher biomass production, lower mortality) of the species tested, coupled with a lower risk of food chain transfer (lower foliar concentrations and herbivory rate). Large leaching losses of heavy metals (particularly Zn and Cd), and low pH may have led to increased pollution of the surrounding canal water. There was some evidence of degradation of organic pollutants but it was slow with respect to other reported methods of remediation such as composting. Low biomass yields and low phytoextraction at the site, together with the low pH of the sediment and the high mobility of heavy metals makes this form of phytoremediation ineffective at this site. It is recommended that the sediment be kept submerged and anoxic until a more viable form of remediation can be proved to be effective.

COLLABORATIVE WORK

This thesis should be considered in conjunction with that of R. F. King, 2005 "Ecological Considerations of Phytoremediating a Contaminated Canal Sediment". Due to the nature of the funding of this PhD, the work detailed in Chapters 7 and 8 were carried out in collaboration with R. King of The University of Liverpool. Collaboration extended only as far as field work and sample analysis.

A placement was also undertaken by the author in CSIRO Land and Water Australia. The work detailed in Chapters 4 and 6 of this thesis was undertaken by the author during this time.

LIST OF ABBREVIATIONS

Abbreviation	Definition
DEFRA	Department of the Environment, Fisheries and Rural Affairs
DGT	Diffusive gradients in thin films
DOC	Dissolved organic carbon
DW	Dry weight
EDTA	Ethylene diamine tetraacetic acid
E_h	Redox potential
EU	European Union
FC	Field capacity
FW	Fresh weight
GF-AAS	Graphite furnace atomic absorption spectrometry
ICP-AES	Inductively coupled plasma – atomic emission spectrometry
ICP-MS	Inductively coupled plasma – mass spectrometry
ICP-OES	Inductively coupled plasma – optical emission spectrometry
LOI	Loss on ignition
MC	Moisture content
no.	Number
OM	Organic matter
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
SD	Standard deviation
SRC	Short rotation coppice
TPH	Total petroleum hydrocarbon
UK	United Kingdom
v/v	Volume to volume ratio
w/w	Weight to weight rato
WHC	Maximum water holding capacity
WNCC	Woolston New Cut Canal

Units	
b	bredth
Bq	Becquerel(s)
cm	centimetre(s)
d	day(s)
g	gram(s)
g	gravity
h	hour(s)
h	height
ha	hectare(s)
kBq	kilo Becquerel(s)
kg	kilogram(s)
km	kilometre(s)
L	litre(s)
l	length
M	molar
m	metre(s)
mg	milligram(s)
min	minute(s)
mL	millilitre(s)
mm	millimetre(s)
mV	millivolt(s)
°C	degrees centigrade
ppm	parts per million
rpm	rotations per minute
t	ton(s)
µg	microgram(s)
µL	microlitre(s)
µm	micrometre(s)
y	year(s)

Note Tree names are abbreviated throughout this thesis. For full latin names see Royle *et al.* (2003) in Appendix 10.

CHAPTER 1

An Introduction to the Woolston New Cut Canal

1.1 Introduction

The field site throughout the duration of the current project was the Woolston New Cut Canal (WNCC), located in Woolston, Warrington, in the North West of England (53° 23' N, 2° 33' W). The total length of the WNCC is approximately 2 km. The experimental site constituted a 150 m stretch of the south bank of the canal site, from Paddington Lock (the western-most lock of the canal) to the first swing bridge. The WNCC site was identified as potentially contaminated land by a DoE survey, carried out in the 1980's. An initial survey of the sediment was undertaken in 1990, confirming that the site was polluted. In 2002, funding was acquired to begin the current project.

In April 2002 the established vegetation was cleared from the 150 m stretch of the south bank of the canal and the site was rotivated. Sediment was dredged from the canal channel in April 2002 to form a planting platform (Fig. 1.1), upon which the experimental willow, poplar and alder trees were planted one month later (May 2002). The platform was divided into 6 experimental blocks. Twelve short-rotation coppice species, hybrids and clones of willows, poplars and alders (Table 1), were then planted on the raised platform in double rows (0.5 m x 0.5 m) of 6 plants of each clone, with 1 m between rows. *Salix* and *Populus* species were planted as pegs, and *Alnus* species were planted as 50-70 cm rooted stock (which was pruned back after establishment). The willow clones planted on the canal came from the nursery at Ness Botanical Gardens, Neston and details of their metal accumulation in this, unpolluted, soil can be found in Nissen and Lepp (1997). Species were randomly allocated within each block and empty "control" plots were included in the design. For list of species used see Royle *et al.* (2003) which is presented as an appendix (Appendix 10) to this thesis.

The sediment was a sandy mud containing elevated concentrations of heavy metals. Samples were taken from the sediment 2 months after dredging. These samples were sent to external

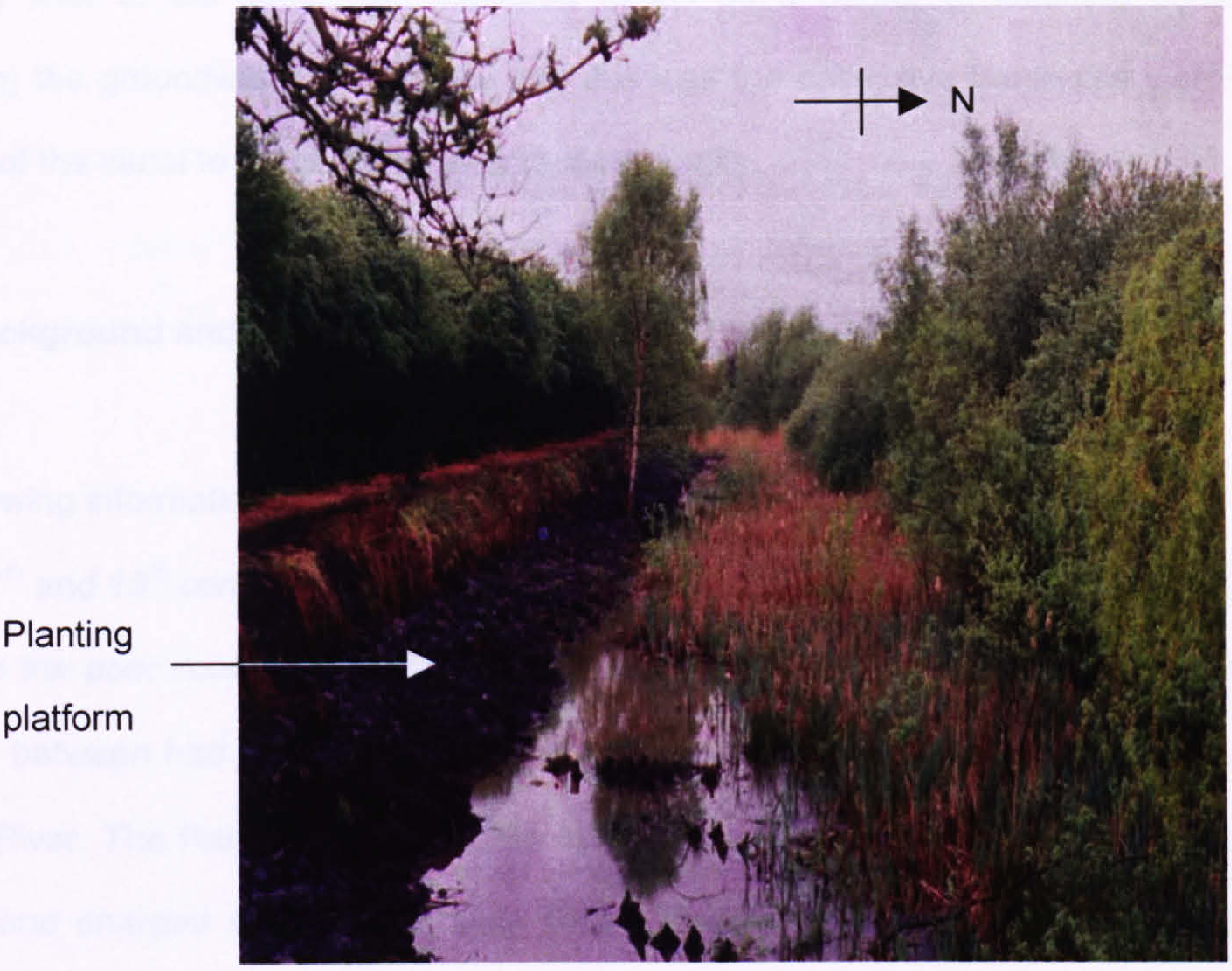


Figure 1.1 The prepared planting platform at the Woolston New Cut Canal, spring 2002, still containing a birch tree, which was removed prior to planting of experimental trees.

laboratories for analysis. The results obtained from the laboratories are given in Appendix 1. The laboratories which carried out the analysis were: 1) ALcontrol Laboratories, Templeborough House, Rotherham, 2) NRM (Natural Resource Management) Ltd., 21-47 High Street, Feltham, and 3) CAS (City Analytical Services) Ltd., 80 Lockhurst Lane, Coventry.

The clay liner of the canal was expected to act as a barrier to leaching contaminants, thus protecting the groundwater. To ensure that this was the case, five boreholes were established to the side of the canal to monitor the groundwater quality.

1.1.1 Background and Site History

The following information in this section is taken from Royle (2002):
In the 17th and 18th centuries, Liverpool was the main port on the River Mersey, being at its mouth. Owing to the poor conditions of the roads, goods travelling from Liverpool to Manchester and the towns in between had to pass through the port of Liverpool, and travel beyond Liverpool via the Mersey River. The Railway was yet to be invented. The port of Liverpool had a monopoly on the Mersey and charged extortionate taxes to all vessels wishing to use the port. Indeed, it was cheaper for a merchant to ship goods half way around the world than it was to pass them through Liverpool.

The Mersey estuary had begun to silt up in the early 1600's, increasingly impeding the progress of boats to Manchester. There were calls for the river to be dredged as early as 1660. However, Liverpool refused to pay for the channel to be dredged as the city did not wish to lose trade to Manchester.

The tidal limit of the Mersey was the bend in the river where the eastern lock of the Woolston New Cut Canal now joins it – a few miles east (upstream) of Warrington. Boats relied on wind and tide to transport them this far, through the many bends of the river, but progress beyond this point was slow and difficult. In 1710 and 1712, Thomas Steers published his idea to dredge and straighten the Mersey so that ships could more easily sail from Bank Quay, Warrington, to Hunts Bank, Manchester. Above the tidal reaches of the canal, a series of weirs, locks and towpaths would be constructed to take ships to Manchester. Work on this plan was slow due to the shallow and winding nature of the river, but began in 1724. By 1734, ships of up to 50 tons could sail to Manchester. This system was still not much used so "new cuts" were created prior to 1740 to make it a more attractive option. Locks were also enlarged and rebuilt. However, the silted up estuary continued to be a problem and in 1804, a canal from Latchford to Runcorn was opened, allowing trade to prosper along the length of the river from Liverpool to Manchester. This prosperity justified the creation of more new cuts to shorten the course of the river still further. In 1821 the Woolston New Cut was created to shorten and widen a difficult section of the old course. In total, the Mersey canals reduced the distance from the Albert Bridge, Manchester to Old Quay, Runcorn, from 38 to 27 miles.

The invention of the steam engine and the proliferation of the railways ended the heyday of canals. Transportation of goods on the railways was not only faster than along the canal system, it was independent of tide and wind. Also the excessive taxes of the port of Liverpool were avoided.

The Mersey was becoming so silted up at the estuary that even flat-bottomed boats could pass only at high tide. The silting led to irregular currents and eddies in the waters and it was not uncommon for the horses which pulled the barges, to be dragged into the river and drowned. The situation was so bad that a radical and seemingly preposterous idea – to build a large canal all the way from Manchester, directly to the Mersey Estuary – eventually won credence. The canal was to be called the Manchester Ship Canal and it was completed in 1893. After the opening of the Manchester Ship Canal, the River Mersey and its many canals became less important but were still active and regularly used to service the towns located between Liverpool and Manchester.

1.1.2 The Woolston New Cut Canal

The Woolston New Cut Canal was opened in 1821. Canals were a good source of water and were convenient for transport of raw materials and finished products. As a result, industries sprang up along their banks. Ordnance Survey maps of the Woolston New Cut Canal show the presence of a "chemical works" on the North bank in 1907, which remained until at least 1937, maybe as late as a decade or two ago; from the maps it is not clear. Between 1907 and 1928 a large tannery occupied part of the south bank of the canal, but this was gone by 1937 and had been replaced by a slaughter house and a tubes and fittings (pipe) works. The pipe works still occupies this site. Thelwall Gunpowder Mill gave the eastern lock of the Woolston New Cut (Powder Lock) its name. However, this facility does not appear on the maps and the duration of its existence is unknown, though it is unlikely to have caused major pollution of the canal as discharge would have consisted mainly of soluble salts. Because of the tidal nature of the river up to this point, it is possible that pollution from the many tanneries, metal works, gas works and other heavy industries from Warrington may have entered the canal when boats were admitted.

During the 19th and 20th centuries, sail power gave way to oil power. Leakage of oil from passing canal traffic and the practice of painting canal boat hulls with coal tar, will undoubtedly have led to pollution of this watercourse. The Woolston New Cut Canal has been disused by water traffic since the mid to late 1940's. During the Second World War it was used for the storage of telegraph poles. The canal flowed until 1978, when the aqueduct across the Mersey was demolished. Since then, the water level has dropped to a maximum level of approximately 1.5 m. The canal channel has become colonised with *Typha latifolia* and *Phragmites australis,* and the banks with sallow (*Salix atrocinerea*) and other tree species such as birch (*Betula pendula*) and understorey species such as nettles (*Urtica dioica*).

1.5 Thesis Aims and Objectives

This thesis examines the Woolston New Cut Canal sediment both as a sediment, and as a medium for the growth of experimental trees. Short rotation coppice (SRC) was established on this site with the aim of phytoremediation of organic and inorganic pollutants within the sediment. This thesis focuses mainly on the contaminants Cd, Cu, Pb and Zn though Ni (Chapter 4) and organic pollutants (Chapter 7) are also included. Arsenic was present in the sediment at elevated concentrations but is not examined within this thesis. The behaviour of As in the WNCC sediment is discussed in detail in the thesis of R. F. King (2005), as are nutrient levels and ecological factors such as colonisation of the sediment by invertebrates.

In the current thesis, the sediment properties and the efficacy of the phytoremediation field trial are examined through a range of aims and objectives (given below).

Objectives

- To investigate the levels of contamination within the WNCC sediment
- To investigate if or how these contaminant concentrations change post-dredging
- To specifically investigate changes in heavy metal behaviour during drying and oxidation of the canal sediment
- To instigate a phytoremediation trial at the WNCC site and monitor its progress over the period of 3 years.
- To evaluate the viability of the phytoremediation trial
- To make recommendations or suggestions for the future management of the site and the pollution therein

Aims

- To examine heavy metal fractionation change during drying and oxidation of the sediment
- To compare these results to other measures of "availability", "lability" or "extractability"
- To relate the measures of extractability to phytoavailability and phytotoxicity
- To find an amendment which could be applied to the sediment to reduce phytotoxicity without negatively impacting on phytoextraction
- To evaluate the efficiency of the phytoremediation field trial
- To isolate factors affecting phytoremediation of the canal site sediment
- To relate experimental laboratory data to the heavy metal behaviour seen at the canal site.

CHAPTER 2

General Introduction to Heavy Metals, Organic Pollutants, Contaminated Land and Phytoremediation

2.1 Heavy Metals

The term "heavy metal" was originally intended to describe those metals with an atomic density greater than that of iron, but is now understood to encompass elements with an atomic density greater than 6 g cm^{-3} or an element with an atomic number > 20 (Alloway, 1995, Phipps, 1981). Arsenic is also accepted as being included in this group, even though it is a metalloid. "Trace elements" is another term widely applied to this group of elements, thus eliminating the arsenic issue, however, the term employed in this thesis will henceforth be "heavy metals".

Many heavy metals are required by biota at trace levels as essential micronutrients e.g. Co, Cr, Cu, Zn (Alloway 1995), serving essential functions such as enzyme co-factors. However, at high concentrations, heavy metals become toxic to biota (Alloway, 1995).

Sources of heavy metals in the environment include:

- Natural sources e.g. weathering of metalliferous bedrock, volcanic activity.
- Industrial sources e.g. smelting plants, release of metals in industrial effluents, mining waste.
- "Domestic" sources e.g. sewage and, previously, lead from the use of leaded petrol.
- Agricultural sources e.g. cadmium applied to soils through phosphate fertilisation.

(Modified from Freedman and Hutchinson, 1981)

The reviews of heavy metals provided by sections 2.1.1 to 2.1.4 have mainly been condensed from Alloway (1995). Information from other sources is referenced accordingly.

2.1.1 Cadmium (Cd)

Cadmium is currently thought to have no essential biological function in plants and animals and is a highly toxic metal. In humans, Cd accumulates in the kidneys and can cause dysfunction if cortex concentrations exceed 200 mg kg^{-1} (FW). Cadmium pollution is an increasing problem, especially in agriculture, where the application of phosphate fertilisers and sewage sludge can add significant amounts of Cd to the soil, but also from industrial sources such as from smelting and mining. Cadmium in soil solution is principally present as the free ion Cd^{2+}, but is also present as complex ions, mainly chlorides and hydroxides, and as organic complexes. Cadmium is more mobile in soils than other metals such as Pb and Cu, due to the lower affinity of some soil constituents for this metal. Soil pH, organic matter and hydrous oxide contents have been shown to be the controlling factors on the specific adsorption (fixation) of Cd. Competition with Ca, Co, Cr, Cu, Ni and Pb ions can inhibit the adsorption of Cd. The presence of EDTA has been found to prevent the adsorption of Cd over the pH range 3 to 11. Under anoxic conditions, Cd forms CdS, which is less bioavailable than oxidised forms of Cd and is thus less of a phytotoxic hazard and also poses less of a threat to human health. Cadmium is readily translocated to plant shoots following absorption by the roots.

2.1.2 Copper (Cu)

Copper is specifically adsorbed or "fixed" by organic matter in soils, making it one of the least mobile heavy metals. The COO^- group, which is prevalent in organic matter, forms stable complexes with Cu. Under oxidising conditions, Cu^{2+} is the most common form of Cu, although under reducing conditions Cu^+ and Cu^0 are more thermodynamically stable. The Cu^{2+} in soils can be chemisorbed onto, or occluded in Fe or Al oxides and silicate clays and also occluded as carbonates. Most Cu in soils with normal levels of organic matter (1 – 8 %) is organically bound, and the combined amounts of water-soluble and exchangeable Cu are generally small (1 – 2 %). Copper in the rhizosphere is almost all organically bound by root exudates or soil humus. Plant uptake is a function of Cu^{2+} activity. The uptake of Cu by plants (along with Zn and P) is modified by the presence of vesicular-arbuscular mycorrhizae. The limiting step of transport of Cu across the plasmalemma involves the electrochemical gradient relating the activity of Cu^{2+} in solution outside the root, to that of the cytoplasm of the cortical cells. Translocation of Cu^{2+} within the plant occurs both in xylem and phloem where the metal is bound by organic nitrogen compounds such as amino acids. In the shoot, N metabolism controls the binding and transportation of Cu^{2+}. Copper is fairly immobile in plants and older leaves may not release Cu to younger leaves, even if they are deficient. Hence, deficiency can be a problem in clean soils. However, Cu exhibits higher phytotoxic than zootoxicity and thus, in polluted soils, Cu induced phytotoxicity may become a problem.

2.1.3 Lead (Pb)

As in the case of Cd, Pb is not an essential element and causes toxicity in adults and mental impairment in children. Lead tends to accumulate in sediments and soils and, due to their low solubility and relative freedom from microbial degradation, lead compounds may remain bioavailable for many years. Lead in soils mainly comes from parent material, mining and smelting, manure and sewage sludge application and historically, anti-knock (Pb alkyl) compounds in leaded petrol. Lead accumulates naturally in surface horizons of soils. The main compartments for lead in soils are the soil solution, the adsorption surfaces of the clay-humus exchange complex, precipitated forms, secondary Fe and Mn oxides, alkaline earth carbonates, the soil humus and silicate lattices. Lead in soil solution is the immediate source for plant roots, but concentrations are usually very low. Thus only a small proportion of total Pb is available for uptake. Uptake and translocation of lead varies seasonally and is dependant on physiological status. Lead may precipitate on cell walls or accumulate at the endodermis and generally only a small to moderate percentage of total lead burden is found in leaf tissues. High soil concentrations of Pb can cause stunted growth or death of plants. Soil pH seems not to be a major factor affecting plant uptake of Pb.

2.1.4 Zinc (Zn)

Zinc is an essential heavy metal for human, animal and plant nutrition. Sources of Zn in soils include parent material, mining/smelting activities, sewage sludge application, volcanic activity and agrochemicals. In natural soils, the concentration of Zn in soil solution is very low (3×10^{-8} to 3×10^{-6} M), however this is much increased in acid soils. Zinc mobility in heavy soils has been shown to be lower than in light or medium soils over a range of pH values. Solution complexes of Zn with phosphate and sulphate are the most important and may contribute significantly to total Zn in solution, though $ZnPO_4$ is perhaps more important in neutral to alkaline soils. The most important binding phases for Zn are clay minerals, hydrated metal oxides and organic matter. Zinc may precipitate as hydroxides, carbonates, phosphates, sulphides, with humates, fulvates and other organic ligands. The forms of Zn taken up by plants are Zn^{2+}, hydrated Zn^{2+} and Zn complexes. Zinc-phosphate antagonism has been known to occur as has antagonism between Zn-Fe, Zn-Cu, Zn-N and Zn-Ca. Zinc acts as a catalytic or structural component in numerous enzymes involved in energy metabolism, transcription and translation. Higher plants predominantly absorb Zn as Zn^{2+}. A deficiency in trees can lead to stunted growth and "little leaf" rosette (malformation of stems and leaves) due to a lack of indoleacetic acid (IAA). Zinc is readily translocated to plant tops after uptake into roots. Zinc-deficient plants express Zn transporter proteins which are involved in Zn^{2+} and Fe^{2+} uptake (Meagher, 2000) and may be inhibited by Mn^{2+}, Co^{2+}, and Cu^{2+}. The Fe transporter protein can also actively and efficiently transport Cd^{2+} and Zn^{2+} into plant roots (Meagher, 2000).

2.2 Organic Pollutants – Petroleum Hydrocarbons and PAH

Human activity has caused large quantities of soil to become contaminated with petroleum products (Boopathy, 2003, Kaimi *et al.*, 2004). Pollution of aquatic sediments is also a problem, for example, in sediments close to petroleum refineries and petrochemical companies (Adeniyi and Afolabi, 2002, Beg *et al.*, 2003), and as the result of oil spills (Lin and Mandelssohn, 1998). Domestic waterways such as canals, rivers and lakes may also be polluted with hydrocarbons that have leaked or been released from water traffic. Petroleum hydrocarbons tend to be aliphatic in nature, with the shorter chain-length hydrocarbons being potentially volatile. On the other hand, polycyclic aromatic hydrocarbons (PAHs) are aromatic in nature e.g. anthracene whose structure is a linear formation of 3 benzene rings (Semple *et al.*, 2001). Creosote is one source of PAH contamination in the environment and is used in petroleum refining and for treating telephone poles (Blakely *et al.*, 2002). Both petroleum hydrocarbons and PAHs are hydrophobic and have a tendency to sorb to soil particles, exhibiting a low rate of desorption (Bonten *et al.*, 1999). Most degradation of these substances tends to be biological and aerobic, owing to the need for an oxidising agent in the early stages of biodegradation (Boopathy, 2003). Therefore, under anoxic conditions, if no electron acceptor is supplied, degradation is unlikely to occur. Throughout this thesis, "organic" pollution with reference to the Woolston New Cut Canal site will refer to pollution with petroleum hydrocarbons and PAH.

2.3 Contaminated Land in the UK and the EU

The following review is provided predominantly by Christie and Teeuw (1998). Information from other sources is indicated:

All European countries have some amount of contaminated land. Those countries that underwent earlier industrialisation are likely to have larger problems than those that underwent later industrialisation. Agricultural practices may also have led to the contamination of rural areas. The formation of the European Union (EU) brought contaminated land awareness and policy to most member states due to the action of "leader" states who "pulled" the rest of the EU forwards on these issues. Denmark and the Netherlands were two of these leader states and they were joined by Austria and Finland upon their entry into the EU in 1995.

Contaminated land policy has focussed on two areas: 1) control of polluting activities and 2) remediation of land which is already contaminated. The aims of the policies also fall into two categories: 1) Safeguarding of human health, the environment and water resources and 2) to conserve land as a resource by preserving soil, re-using Brownfield land and setting soil quality standards. All member states of the EU have followed the "polluter pays" principle, though the actual meaning of this varies between states and in some states substantial amounts of public money fund these remediation projects e.g. Germany after reunification.

Generally member states have their own legislation controlling contaminated land and remediation issues due to the lack of a specific European law on the subject. In England for example, the Environmental Protection Act 1990 Part II A is the main contaminated land policy instrument. This part of the Act became law on 1st April 2000 providing, for the first time, a legal definition of contaminated land and a new regulatory regime for its identification and remediation (Freegard, 2001). The definition of contaminated land given in this act is that, by reason of substances in, or under the land that:

- Significant harm is being caused to specified receptor (targets) or
- Significant pollution of controlled waters is being, or is likely to be caused

This definition was amended to include "significant water pollution" once section 86 of the Water Act 2003 came into force (DEFRA 2004). The legislation identifies the s*ource, pathway, receptor* linkage and states that all three must be present in order for the land to be posing a significant threat. Local authorities are given responsibility for identifying contaminated land in their administrative area. The Act also introduced a "Contaminated Land Remediation Register" (Freegard, 2001), meaning that the UK was no longer the only member state in Europe lacking one.

The UK has chosen to utilise the BATNEEC (Best Available Technology Not Exceeding Excessive Cost) principle to remediate contaminated land, whereas some member states prefer BAT (Best Available Technology). BAT, though potentially more expensive, is more likely to produce sustainable development and tends to lead to remediation of the land to "multifunctionality", where contaminants are decreased to background levels. BATNEEC is applied to remediate land to a "suitable for use" site and the extent of remediation will depend on the end use of the site. Its purpose is to cut the source to target pathway.

Soil standards and guidelines differ between member states. The Dutch C-values which have been extensively referenced across the world were designed for standard Dutch soils (25 % clay, 10 % organic matter) and do not claim to have any validity for other soil types, though this has been much ignored by other nations. In addition, such guidelines are not comprehensive. For example, the UK ICRCL (Interdepartmental Committee on the Redevelopment of Contaminated Land) trigger values contained guideline values for only 10 metal(loids) and took no account of the chemical form of the element. The Dutch list was probably the most comprehensive in Europe but still did not cover the full range of contaminants. In the UK, the ICRCL guidelines (set out in the document ICRCL 59/83) were replaced in December 2002 by the CLEA (Contaminated Land Exposure Assessment) computer model (DEFRA 2002) which is intended as a generic assessment of direct risks to human health. Where computer generated values are exceeded, further risk assessment or remedial action will be triggered. However, the future of these guidelines may perhaps be uncertain. Much risk assessment and remediation work is undertaken in response to planning controls. Many contaminated sites have planning restrictions on them and in order to remove or reduce these, remedial work must be carried out. If the site has a financially profitable end-use, then remediation will be economically viable.

2.4 Phytoremediation and Bioremediation

If a contaminated site is not of high economic value and/or the polluter is either untraceable or no longer in business, the cost of the remediation may be too great to justify the physical or chemical methods available such as soil washing (Sikdar *et al.*, 1998), excavation, incineration, electrokinesis (Acar, 1993, Virkutyte *et al.*, 2002), microwave heating (Jones *et al.*, 2002) and air sparging (Bass *et al.*, 2000). For general reviews see Hamby (1996) and Mulligan *et al.* (2001a), Mulligan *et al.* (2001b). A cheaper option may then be investigated. Bioremediation is the practice of using living organisms to aid remediation of contaminants. Though the term "bioremediation" covers all forms of biological organism it is associated more with the utilisation microbial species for the remediation of both organic and metal contaminants. This subject has been the focus of many review articles such as those by Pollard *et al.* (1994), Wilson *et al.* (1993), Romantschuk *et al.* (2000) and Khan (2005). Composting can be a useful and relatively cheap technology for the remediation of organic pollutants and explosives (see review by Semple, 2001). Other alternatives include the exploitation of oxidative and extra-cellular enzymes (Durán and Esposito, 2000, Gianfreda and Rao, 2004). Phytoremediation is generally used separately from the term "bioremediation" and refers specifically to the use of plants to help to remediate both organic and metal(loid) pollution. The major limitations on phytoremediation, compared to chemical remediation methods, are that plants are living organisms and will only thrive in conditions which supply them with adequate light, water, oxygen, nutrients and appropriate soil conditions (Cunningham *et al.*, 1995). Exploitation of mycorrhizae to increase metal uptake during phytoremediation has been explored (Meharg and Cairney, 2000, Khan *et al.*, 2000). General reviews of phytoremediation are provided by Cunningham *et al.* (1995), Ernst (1996), Khan *et* al. (2000), Macek *et al.* (2000), Alkorta and Garbisu (2001), Römkens *et al.* (2002) Williams (2002), McGrath and Zhao (2003), Pulford and Watson (2003), Khan (2005), Krämer (2005), and Meagher (2000). Phytoremediation can be arbitrarily divided into two main groupings – the use of hyperaccumulating and non-hyperaccumulating plant species. A short summary of the two divisions follows. Though the principals of phytoremediation may apply to both, only the principals of phytoremediation using non-hyperaccumulating plants are discussed in detail.

2.4.1 Hyperaccumulation

Hyperaccumulation of metals by plants is a rare phenomenon and occurs in < 0.2 % of angiosperms (McGrath and Zhao, 2003). Hyperaccumulation is generally defined as being the accumulation by a plant of metal ions equivalent to > 0.1 – 1 % of its own dry weight (Meagher, 2000). The difference is segregated by metal, with hyperaccumulation defined as accumulation of 10 000 mg kg^{-1} dry weight of shoots for Zn and Mn, 1000 mg kg^{-1} of Cu, Co, Ni, As and Se and 100 mg kg^{-1} of Cd (McGrath and Zhao, 2003). At these concentrations, the recovery of metals from the plant is potentially economical (Meagher, 2000) in the form of "phytomining" (McGrath and Zhao, 2003). Hyperaccumulators have been used as bio-indicators of mineral deposits for more than a century. The majority of hyperaccumulating plants are from the family Brassicaceae (Meagher, 2000). In some hyperaccumulator species (e.g. *Alyssum lesbiacum)*, chelation with histidine, uptake, xylem transport and hyperaccumulation (of nickel and cobalt) appear to be mechanistically linked, whereas in other species (e.g. *Thlaspi goesingense*) histidine may only be involved in transport of nickel and zinc from the rhizosphere to the root (Meagher, 2000). Metallothioneins and phytochelatins also sequester metals and may be involved in hyperaccumulation in some species

(Meagher, 2000). For a review of molecular mechanisms of heavy metal hyperaccumulation and phytoremediation see Yang *et* al. (2005). The list of hyperaccumulators is still growing. The brake fern *Pteris vittata* was reported in 2001 to hyperaccumulate As at levels of up to 22 000 mg kg^{-1} and more recently several other ferns have also been reported as being As hyperaccumulators (McGrath and Zhao, 2003).

Hyperaccumulating plants generally produce low biomass e.g. *Thlaspi caerulescens* which typically produces 2 – 5 t ha^{-1} of shoot dry matter. However, fast-growing, high biomass hyperaccumulators do exists e.g. the Ni hyperaccumulator *Berkheya coddii,* (22 t ha^{-1}) (McGrath and Zhao, 2003). These high-biomass hyperaccumulators have potential for phytomining activities in the future if they can be grown effectively on potential mining sites. At the present time there is a lack of confirmed high-biomass hyperaccumulators of Cu, Pb and Cr (McGrath and Zhao, 2003). A problem with hyperaccumulation is that contaminated biomass, such as leaf fall, needs to be removed from the site before it is decomposed and the metals re-released. Transfer of metals to the food chain may also be an issue if the plants are eaten by herbivorous animals.

2.4.2 Phytoremediation Using Non-Hyperaccumulator Species

The rate of accumulation of metals in non-hyperaccumulator plant species grown on uncontaminated soils may be two or three orders of magnitude lower than those concentrations found in hyperaccumulating plants (McGrath and Zhao, 2003). However, the greater range of species available and their higher biomass production, make them feasible options for phytoremediation. Phytoremediation is divided into five categories:

- Phytostabilisation – the use of plants and associated rhizosphere effects to precipitate pollutants from solution, or absorb/entrap them in either plant tissues or the soil matrix (Cunningham *et al.*, 1995).
- Phytoextraction – using plants to extract contaminants from the soil/water. Plants may be harvested so that contaminated biomass is removed from the site (Cunningham *et al.*, 1995).

- Phytodegradation – the presence of a rhizosphere can increase microbial activity within the soil, increasing the rate of degradation of organic contaminants into non-toxic materials (Cunningham *et al.*, 1995). Polycyclic aromatic hydrocarbons and petroleum hydrocarbons are particularly rapidly degraded (Macek *et al.*, 2000).
- Phytovolatilisation – when plants transform contaminants into volatile compounds (Krämer, 2005), thus removing the pollution from the soil.
- Rhizofiltration – the use of plant root to absorb and adsorb pollutants, mainly metals, from water and aqueous waste streams (Macek *et al.*, 2000).

Phytoremediation utilising hyperaccumulating species tends to focus on phytoextraction.

There have been many studies on the uptake and/or effect of metals on wetland plant species or vice versa e.g. Stoltz and Greger (2002), Deng *et al.* (2004), Jacob and Otte (2004), Matthews *et al.* (2005), and of the use of wetland plants for site phytoremediation (see the review by Weis and Weis, 2004). In the present study, the phytoremediation potential of planted trees, rather than naturally occurring wetland species, is being examined. In the following short review, the role of *Salix*, *Populus* and *Alder* species are examined owing to their relevance to the current project.

Madejón *et al.* (2004) found that Cd and Zn concentrations in leaves of poplars growing on a site contaminated by a mine spill, were positively and significantly correlated with soil availability. Concentrations of Cd, Cu and Zn were also elevated in the poplar stems with respect to the control trees. Laureysens *et al.* (2005) found significant differences in biomass production and phytoremediation potential between six poplar clones in SRC (short rotation coppice) over a two-year growing period, highlighting the importance of genotype in phytoremediation.

Poplar trees have been shown to successfully decrease concentrations of organic contaminants in soils and water (Wang *et al.*, 2004, Widdowson *et al.*, 2005). However, Widdowson found that remediation was limited to naphthalene and several three-ring PAHs. The extent of remediation of these compounds was limited by their rate of dissolution from the source. TPEM (two-photon excitation microscopy) has recently been used to show that the PAH anthracene translocates from the waxy cuticle of maize plant roots, into the cell cytoplasm, despite having a relatively low solubility in water (Wild *et al.*, 2005). Wild concluded that movement of anthracene within the maize roots is dominated by apoplastic flow, but that a low level of symplastic flow to the cellular vacuoles is also present. Degradation of the compound was also witnessed within the cortex cells. Meagher (2000) reports that the glutathione-S-conjugate pump can recognise glutathione conjugates of organic pollutants and thus they can be transported into cell vacuoles, where their subsequent degradation should be favoured. The aim of phytoremediation of organic pollutants is complete mineralisation to CO_2 and H_2O (Meagher, 2000), though this may not always be fast and efficient. Huang *et al.*, (2005) combined land farming and bacterial additions with phytoremediation and found a 100 % increase in TPH (total petroleum hydrocarbon) degradation compared with phytoremediation alone.

Short rotation coppicing (SRC) of willow (*Salix*) species is potentially a viable source of biomass fuel (Perttu, 1999, Perttu *et al.*, 1997, Vandenhove *et al.*, 2001) and can be combined with phytoremediation as long as the ash and fumes created by incineration are treated appropriately (Krämer, 2005). Keller *et al.* (2005) confirm that incineration of heavy metal contaminated biomass can successfully separate out the heavy metal pollutants. Vandenhove *et al.* (2001) examined SRC on radiologically contaminated land and concluded that energy production by this technique was both radiologically and economically sustainable. Willow coppice is also a good habitat and provides enhanced biodiversity in comparison to coniferous plantations or agricultural crops (Perttu, 1999). Biomass energy from SRC is more environmentally justifiable than the burning of fossil fuels as it is a sustainable resource which locks carbon into its biomass during growth and releases it again upon combustion and thus is said to be CO_2 neutral (Börjesson, 1999, Perttu, 1999). The carbon locked away by fossil fuels millions of years ago increases the amount of CO_2 in the present day atmosphere during combustion and hence is not CO_2 neutral in today's world. Willow coppice is practiced in Sweden for biomass energy production, phytoremediation and organic waste filtration e.g. municipal wastes, sludges and landfill leachate (Mirck *et al.*, 2005, Perttu, 1999). Phytoremediation can overlap with biomass energy production or production of solid wood/reconstituted wood products, serving a duel purpose (Licht and Isebrands, 2005, Mirck *et al.*, 2005). Dickinson and Pulford (2004) argue that remediation of land contaminated with Cd could be achieved within a realistic crop lifecycle using *Salix* clones.

Populations of the same *Salix* clone growing in contaminated compared to uncontaminated areas do not seem to show intrinsic metal tolerance or uptake abilities (Landberg and Greger, 1996), suggesting that their ability to withstand metal-polluted soils is acquired throughout their lifetime. However, when clones with known higher resistance were compared to clones with known lower resistance to heavy metals, the more resistant clones were found to have a higher intrinsic activity of superoxide dismutase in non-stressing conditions (Landberg and Greger, 2002). The superoxide anion (a free radical) can be generated within a plant by the presence of heavy metals. It is a very aggressive species and the plant produces superoxide dismutase to convert it to hydrogen peroxide, which is then converted to water by catalase (Landberg and Greger, 2002). Therefore, the more resistant clones were more resistant to free radical attack than the less resistant clones, giving them a competitive advantage when grown on contaminated soils. Punshon *et al.* (1995) also note differences between willow clones, in this case for copper resistance. Bioavailability of metals to trees and hence metal accumulation in tree tissues also varies from site to site, depending on site conditions and the origin of the contamination (Pulford and Watson, 2003). Thus the results gained from studies on phytoextraction of metals from soil polluted by mining activity, smelting activity, agricultural activity and sediments containing pollutants are likely to different. However, some similarities probably can be drawn.

Phytoremediation of dredged sediments may be a viable option both for biomass production (for biomass energy) or simply for creating biodiverse coppice stands. *Salix* species have been shown to grow well in contaminated dredged sediments despite their high moisture content (Vervaeke *et al.*, 2001), with an average productivity of 13.4 t ha^{-1} y^{-1} (DM). Meers *et al.* (2005) found that, despite good biomass production on contaminated dredged sediments (13 – 18 t ha^{-1} y^{-1}), phytoextraction of elements was low. However, the clone used by these authors was not a known accumulator of heavy metals. The sediment was also calcareous. Willow species have shown potential as phytoextractors on sediment-derived soils (Vandecasteele *et al.*, 2005) and also for phytodegradation (Vervaeke *et al.*, 2003). When alder and poplar species were planted on a brackish, dredged, slightly polluted sediment, the poplar (*Populus alba*) showed good growth but growth of the alder (*Alnus glutinosa*) was stunted, though heavy metal levels within the foliage were not elevated (Mertens *et al.*, 2004). The poplar accumulated high levels of metals but the time scale for the treatment to be effective meant that it was not a viable option for phytoextraction (Mertens *et al.*, 2004), and the potential for food chain transfer was also increased.

2.4.3 Genetic Modification

Genetic modification techniques have the potential to increase a plant's resistance to the toxic effects of heavy metals (Bittsánszky *et al.*, 2004), increase the (hyper)accumulating potential of a species (Thomas *et al.*, 2002, Bañuelos *et al.*, 2005) or to allow a species to gain new traits such as the ability to take up and volatilise organic pollutants (Krämer, 2005). For a review of biotechnological advances, see the review by Eapen and D'Souza, 2004.

2.5 Canals and Canal Sediments

At their peak, British canals had a total length of more than 6000 km and were used to transport more than 40 million tonnes of freight per annum (Sidaway *et al.*, 1995). Industries commonly became established alongside these canals and, though many of these industries have now gone, pollution from many years of wastewater discharge and leakage from canal barges may persist. Sediments may remain polluted for decades after the pollution input has ceased. These polluted sediments may be dredged and deposited onto land, either for remediation purposes or in order to keep the waterways open for navigation. Dredging of sediments causes changes in their redox state (Caille *et al.*, 2003), as the initially reduced sediment is exposed to an oxidising environment. This redox change can cause changes in metal speciation and availability (Guo *et al.*, 1997, Stephens *et al.*, 2001a) and also pH, as the sulphides present can oxidise to sulphates (including H_2SO_4) and other intermediary oxidised sulphur compounds (Tack *et al.*, 1997). Consequently, sediments with low carbonate content and low acid buffering capacity may undergo significant acidification (Gambrell *et al.*, 1991). However, the release of metals can be small as long as the pH remains neutral or slightly alkaline (Flyhammar and Håkansson, 1999). Hence the resultant pH and metal release will be a function of oxidisable S content, metal content and pH buffering capacity, with the latter being a function of carbonate and clay content/type. Thus the potential pH decline and metal release for any sediment will be highly dependant on its chemical composition.

CHAPTER 3

Heavy Metal Fractionation Change During Drying and Oxidation of a Dredged Canal Sediment

3.1 Introduction

When sediments are removed from their anoxic environments by processes such as dredging and are disposed of onto land, such as has happened at the Woolston New Cut Canal Site, they are exposed to an oxidising environment. This change in redox conditions may alter the speciation of heavy metals within the sediment. This chapter describes a fractionation study that was applied to the Woolston New Cut Canal (WNCC) sediment. Fractionation studies yield vital information about the strength with which heavy metals are bound in the sediment system and hence, how likely these metals are to change speciation and/or mobility, should chemical conditions change. This information is of particular significance as it may allow prediction of the viability of the WNCC phytoremediation field trial. To the author's knowledge, this study is novel owing to the use of: an anoxic sequential extraction procedure, a highly organic sediment which undergoes natural acidification upon oxidation and a time series examining the very early to medium-term metal fractionation changes at different depths, (particularly in the top 1 cm) of the sediment.

3.1.1 Fractionation Studies

Total heavy metal concentrations in soils and sediments are not a good indicator of potential metal mobilisation and environmental availability (Tessier *et al.*, 1979, Davidson *et al.*, 1994, Clark *et al.*, 2000, Gleyzes *et al.*, 2002). An understanding of the partitioning of heavy metals throughout a soil or sediment allows estimations of present and potential future mobility to be made (Clark *et al.*, 2000). Sequential extraction (also referred to as sequential leach, selective extraction, or fractionation) procedures are utilised to study this partitioning/fractionation. Perhaps the most well known of these was devised by Tessier *et al.* (1979), for bottom sediments and suspended particulate material. Tessier identified 5 fractions with a mind to the effects of changing environmental conditions. Most sequential extraction procedures base their extractable phases on this model. The phases/fractions are:

1. **Exchangeable** – those metals likely to be liberated by changes in water ionic composition.
2. **Bound to carbonates** – a fraction susceptible to pH change.
3. **Bound to Fe and Mn oxides** – thermodynamically unstable under anoxic conditions.
4. **Bound to organic matter** – oxidising conditions lead to breakdown of organic matter.
5. **Residual** – mainly primary and secondary minerals, some more resistant organic matter and sulphides.

Tessier states that "[t]hese [residual] metals are not expected to be released in solution over a reasonable time span under the conditions normally encountered in nature" and hence this fraction was not quantified in this study.

Many subsequent studies on metal fractionation in soils and sediments have employed the selective sequential extraction procedure of Tessier *et al.* (1979), or a modification of it (e.g. Gauthreaux *et al.*, 1998, Zoumis *et al.*, 2001, Pagnanelli *et al.*, 2004). Others have used different procedures to access similarly defined fractions (e.g. Davidson *et al.*, 1994, Guo *et al.*, 1997, van Ryssen *et al.*, 1999, Clark *et al.*, 2000, Stephens *et al.*, 2001a + b). Many different sequential extraction procedures exist because no extractant is absolutely specific to a particular pool of metals (Clark *et al.*, 2000) and hence, each fraction extracted is said to be "operationally defined" by extractant and procedure (Davidson *et al.*, 1994, van Ryssen *et al.*, 1999).

3.1.2 Anoxic Sediments

In waterlogged sediments, where biological activity depletes molecular oxygen and generates more electrons than can be "mopped up" by available electron acceptors, conditions become reduced and the redox potential (E_h) decreases. In general, the higher the organic matter content of a sediment, the more rapidly this decrease in redox potential will progress. The reduction process is possible because the biological activity depletes molecular oxygen at a higher rate than it can be resupplied, due to its low solubility (8 µg mL^{-1}) (McBride, 1994).

Under anoxic conditions, organic matter breakdown will slow significantly, sesquioxides will be reduced at E_h values of 50 to –500mV (van Ryssen *et al.*, 1999), and sulphides will begin to form at E_h –130 mV and below (Guo *et al.*, 1997). When the sediment is exposed to an oxidising atmosphere, such as after dredging, these processes will be reversed: organic matter will be broken down more rapidly, potentially releasing heavy metals previously bound within their structures; sulphides will oxidise to form sulphates and intermediary oxidised sulphur compounds (Tack *et al.*, 1997), releasing metal sulphates into solution; sesquioxides will form, potentially co-precipitating heavy metals, and providing a large surface area for sorption. Thus, heavy metal fractionation would be expected to change as the available binding phases change during reduction or oxidation.

Sediments have often been the subject of fractionation studies. However, it is not only the extraction methods which vary, but sample handling techniques differ also. For example, sediments are sometimes dried before extraction (Tessier *et al.*, 1979, Stephens *et al.*, 2001a, Gauthreaux *et al.*, 1998). This process increases homogeneity of the sediment and makes it a simpler substrate to work with. However, if the sediment was anoxic when sampled, drying would alter the redox potential of the sediment and may cause chemical changes (Clark *et al.*, 2000). Gambrell *et al.* (1991) have reported that changes in redox potential of an anoxic sediment can significantly affect the solubilities of Cd, Ni, Pb and Zn, even when no change in pH occurs. It is therefore logical to assume that the results obtained from extraction of a dried sediment, are those for an oxidised or semi-oxidised sediment and thus do not necessarily accurately reflect the fractionation which would be found in the anoxic sediment. Furthermore, it is important to understand metal behaviour during oxidation, if dredging of a contaminated sediment is to be performed.

3.1.3 Aims

The aims of the current chapter were:

- To examine and quantify heavy metal and Fe fractionation change in the WNCC sediment as it oxidised in air.
- To use this information to comment on any possible implications for the phytoremediation field trial.

3.1.4 Hypothesis

Oxidation of the sediment will lead to the redistribution of heavy metals from the less available sulphide phase into more available phases as the sulphides oxidise. It is expected that this will result in a flush of soluble heavy metals (as metal sulphates), which will then redistribute into the less available phases via the natural sediment equilibrium processes.

3.2 Materials and Methods

3.2.1 Sediment Collection and Storage

Sediment was dredged from the Woolston New Cut Canal field site on 30th January 2003 and stored in closed black dustbins, covered with a layer of native canal water, for 12 d until the start of the experiment. Remaining sediment for use in further experiments, was stored in a large barrel, covered with a layer of native canal water, with a lid firmly in place to avoid contamination.

3.2.2 Tank Pre-Preparation and Sediment Addition

Twelve identical 'Hyware' 32.5 x 22.5 x 20 cm (*l* x *b* x *h*), plastic tubs were acid washed and labelled from 1 to 12. A hole of 5 mm diameter was drilled in each of the short sides at 5-10 mm height from the tub bottom, to allow leachate outflow. Leachate collection tubes were then attached and bunged. A 2 cm layer of acid-washed gravel was put into the bottom of each tub. On top of this was laid a 1.5 cm thick layer of damp, acid-washed sand (Fig. 3.1).

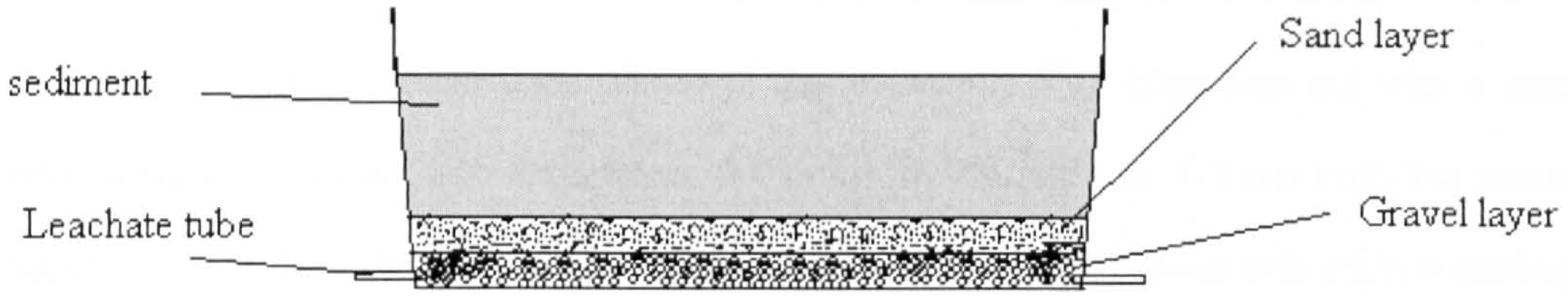


Figure 3.1. A representation of the tubs of sediment (not to scale).

Moisture content of the sand was maintained with deionised water, so that it remained in layer and did not fragment and fall into the gravel layer. (Both sand and gravel were rinsed thoroughly with distilled and deionised water after acid-washing, to remove all traces of acid).

Before addition to the tubs, the sediment was thoroughly homogenised. Large pieces of debris, and root masses were removed by hand. Approximately 7 L of sediment was then added to each of the 12 pre-prepared tanks. The sediment was poured onto a piece of angled Perspex held above the sand surface to reduce its velocity, so that the sand was not damaged, or the layer distorted, upon addition of the sediment. The sediment in each tank was then individually homogenised and rechecked for debris. Initial samples were taken from each tub and pooled, to be the “0 days” sample. The sediment surface was then flattened, for uniform surface area, and the tanks were left in a greenhouse to allow the sediment to dry and oxidise. The average daytime temperature in the greenhouse over the experimental period was 14 ± 5 °C and the average night time temperature was 10 ± 5 °C.

3.2.3 Sediment Sampling

Excluding the initial sampling described above, four tubs were sampled at each sampling event. Tubs were selected for sampling by number, using the random number generator of a hand-held calculator. Cores were taken using lengths of 4.5 cm diameter plastic drainpipe, which had been halved and rejoined with tape. After coring, a rolled acetate, cut to an appropriate height, was inserted into each hole and gently pressed against the sides of the sediment to prevent oxidation of the newly exposed sediment.

In the laboratory, the tape at one side of the soil corer was cut with a scalpel. The corer was opened carefully so as to avoid disturbance of the sediment. The core was cut with a sectioning knife, as quickly as possible, into 3 sections: 0-1 cm from the surface, 1-3 cm from the surface and 3 cm depth to the bottom of the core (3-x cm). Each section was delivered into a small polyethylene bottle (30 mL for the first section and 60 mL for the other two sections), rapidly homogenised, and the bottle was then purged with N_2 gas and sealed to avoid oxidation during storage. Core sectioning was not carried out in a N_2 atmosphere, as Tack *et al.* (1996) report that exposing an anoxic sediment to air for a short period, and then returning it to anoxic conditions, had no significant effect on metal behaviour.

Due to sediment shrinkage during drying, the final depth of the sediment changed with time and the final section is henceforth labelled as "3-x cm". Also owing to this shrinkage, the sections cut did not necessarily constitute the same material each time.

Whenever sample bottles were opened for subsampling of the contents, they were purged with N_2 gas and sealed again immediately afterwards. All sediment samples were stored at 4 °C.

3.2.4 Chemical Analysis

Dry weight was determined by drying separate subsamples of sediment at 105 °C for 3 days. Loss on ignition was carried out at 480 °C for 8 hours. All samples were replicated at least twice. Field capacity of the sediment was determined by the method described by Jenkinson and Powlson (1976). Sediment pH was measured in a 1:5 sediment-to-water suspension, which had been shaken for one hour and allowed to settle for half an hour. Carbonate content was determined using a rapid titration method, modified from Allen (1989) where 10 mL of 0.5 M HCl was added to 1 g dry weight equivalent of sediment. Back-titration was with 0.5 M NaOH. Carbonate content measurement for all samples was carried out in duplicate. For the later samples titrated in this manner, 5 mL of acid was used, as reaction was found to be negligible.

3.2.5 Sequential Extraction

The sequential extraction procedure used was modified from that described by Clark *et al.* (2000). Clark also described a grainsize normalisation procedure where the < 63 µm fraction was isolated, and that alone is used for the extraction. In the current experiment, grainsize normalisation was not undertaken as the aim of the thesis is to characterise the sediment as a whole and to use this information to examine the viability of phytoremediation of the site. Therefore, to use one size fraction of the sediment only, would not have given an accurate representation of the bulk sediment. Further evidence of this is provided by the work of Pagnanelli *et al.* (2004) and Table 3.1. Table 3.1 gives the particle size range of each fraction isolated, its percentage abundance in the WNCC sediment and the heavy metal concentration (mg kg^{-1}) within that fraction. These data represent the sediment 8 months after dredging. The data show that for all of the metals presented, similar concentrations of heavy metals existed in all size fractions of the sediment. The < 63 µm fraction made up only 12.9 % of the total sediment volume. Different size particles tend to represent different binding phases with clay particles mostly found in the < 63 µm fraction. This is often where the majority of heavy metals are also found. However, perhaps due to the highly organic nature of the sediment, appreciable proportions of the total heavy metals with in the sediment were found in all size fractions.

All of the extractants used in this procedure, with the exception of the HNO_3 and the H_2O_2, were thoroughly purged with N_2 gas before each use.

Table 3.1 The percentage abundance of, and total heavy metal concentrations in, the different size fractions of the WNCC sediment. Sizes were separated by sieving of air dried, ground sediment. Values are mean (SD), ($n = 3$). Analysis of variance showed that for all metals, with the exception of Pb, the 1000 – 2000 µm and 500 – 1000 µm size fractions contained significantly higher concentrations of metal than did the fractions in the range 63 – 500 µm ($p < 0.001$ for all except Cd where $p < 0.005$). Total lead was significantly higher in the 1000 - 2000 and < 63 µm fractions than in the fractions ranging from 63 to 250 µm ($p < 0.001$).

Particle size (µm)	**%**	**Heavy metal (mg kg^{-1})**					
		Fe	**Cd**	**Cr**	**Cu**	**Pb**	**Zn**
1000- 2000	19.5	**51549** (2102)	**9.63** (0.75)	**880** (28)	**653** (20)	**1334** (25)	**2500** (81)
500-1000	22.1	**47341** (2244)	**9.31** (0.77)	**825** (78)	**603** (56)	**1257** (114)	**2490** (174)
250 - 500	21.2	**39887** (1715)	**7.51** (0.52)	**660** (54)	**474** (40)	**1042** (51)	**1959** (147)
125-250	15.2	**40511** (737)	**7.91** (0.28)	**705** (30)	**490** (22)	**1108** (23)	**2054** (76)
63-125	9.2	**41403** (321)	**8.10** (0.12)	**730** (22)	**501** (16)	**1178** (37)	**2126** (62)
< 63	12.9	**47404** (1133)	**8.88** (0.25)	**823** (30)	**544** (22)	**1327** (39)	**2349** (112)

Steps 1 & 2 Pore Water and Exchangeable Metals

Step1

For each sample, sediment equivalent to 0.5 g dry weight, was weighed into a 50 mL centrifuge tube. The headspace of the tube was purged with N_2 gas, and the tube was sealed to prevent ingress of oxygen. Once sediment for the whole run had been weighed out, 20 mL of deionised water was added to each tube. Headspaces were purged with N_2 gas and each tube was then manually shaken to disperse the sediment. Samples were shaken at 55 rpm for 1 h on an end-over-end shaker. After shaking, samples were centrifuged at 3000 *g* for 20 min. Supernatant was filtered through Whatman no. 542 filter papers, retained, and acidified with nitric acid. The headspaces of the centrifuge tubes containing the sediment plugs were purged with N_2 immediately after decantation of the supernatant. At the end of this step (Step 1) only, the sediment plugs were frozen to halt the extraction process. Freezing of anoxic sediment samples, unlike drying, will not greatly alter chemical speciation before or during a sequential extraction process (Clark *et al.*, 2000).

Step 2

The sediment plugs were defrosted on the bench top after overnight freezing. When defrosted, 20 mL of 0.1 M NH_4Cl (ammonium chloride) was added to each tube and they were then treated as in Step 1, after the addition of the extractant. The supernatant solution was combined with that obtained during Step 1. Metals present in this combined extract are henceforth referred to as **"NH_4Cl-extractable"** and represent the pore water and exchangeable metals.

Step 3 Carbonate Bound and Adsorbed Metals

The extractant used in this step was a 1 M sodium acetate (NaOAc) solution, buffered to pH 5 with acetic acid (HOAc). Twenty mL of this solution was added to each tube and they were then treated as in Step 1. The supernatant was acidified and retained for metal analysis and is henceforth referred to as the **"NaOAc-extractable"** fraction.

Step 4 Reducible (Fe and Mn Oxide Bound)

The extractant used in this step was an equal v/v mixture of a 30 g L^{-1} sodium dithionite solution and a 90 g L^{-1} tri-sodium citrate solution. A total of 20 mL of the combined solution was added to each tube. Tube headspaces were purged with N_2, tubes were sealed and then manually shaken to break up the sediment plug. Samples were then shaken for 16 h on an end-over-end shaker at 55 rpm. After shaking, samples were filtered and centrifuged as described above, except that the supernatant was not acidified. Acidification of the reducing dithionite solution with an oxidising acid such as HNO_3 caused massive precipitation, and hence was avoided. Metals from this step of the extraction are henceforth referred to as **"dithionite-extractable"**. The solids were retained for microwave digestion.

Step 5 Oxidisible (Sulphide and Organic Matter Bound)

Originally this step was to be a 3:1 HNO_3:HCl microwave assisted digestion. However, due to the highly organic nature of the sediment and, in most cases, its reduced nature, resistant flocculates appeared in the acid extracts whilst they were awaiting analysis. The use of H_2O_2 in place of HCl, and a digestion program of increased temperature and duration (as directed by the microwave manufacturer) eliminated this problem.

The sediment was washed out of the centrifuge tubes into Teflon microwave digestion vessels with 10 mL concentrated HNO_3. A further 3 mL of 30 % H_2O_2 solution was added to each, and the samples were left to pre-digest for 15 min, or until reaction had subsided. Samples were then digested in a MARS 5 (Microwave Accelerated Reaction System) microwave digester (CEM Corporation) at 210 °C for 30 min, after a 10 min ramp to that temperature. Digested samples were filtered through Whatman no. 542 filter papers and made up to 25 mL with deionised water. Metals extracted in this step are henceforth referred to as **"HNO_3/H_2O_2-extractable"**.

The solids remaining after this digestion were not saved for any analysis of residual metals (i.e. those in residual silicates and oxides and also those in the mineral matrix).

Metals in all extracts were analysed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Perkin Elmer Optima 3300 RL). Due to high sodium concentrations in the extractant solutions, NaOAc and dithionite extracts had to be diluted 10 times before analysis.

Total Heavy Metal Content

Total heavy metal content was determined by carrying out the microwave-assisted digest described above. This procedure was carried out on samples of sediment that had not been extracted in any way prior to digestion.

3.2.6. Statistical Analysis

Statistical analysis was carried out using the SPSS v11 statistical package. Kruskall-Wallis analysis was used, as was the General Linear Model (GLM) and Mann-Whitney U tests where appropriate. Quoted p-values are derived from these tests.

3.3 Results and Discussion

There is a lack of data concerning fractionation changes in canal sediments during the very early stages of oxidation. Some authors have analysed sediment depth profiles (Stephens *et al.*, 2001a, Zoumis *et al.*, 2001) or distance-from-source or multiple-site studies (Jones and Turki, 1997, Clark *et al.*, 2000), or studied metal solubilities during oxidation (Gambrell *et al.*, 1991, Tack *et al.*, 1996, Guo *et al.*, 1997, Caille *et al.*, 2003). The study conducted by Stephens *et al.* (2001 b), is perhaps the most similar to current study. However, the first time point in their study was after 2 weeks and the top 2 cm of the sediment were examined. Perhaps the most novel feature of the current study is that the fresh-water sediment undergoes significant natural acidification during oxidation. Tack *et* *al.* (1996) experimented with artificial acidification but this is unlikely to accurately predict actual changes which would occur during natural acidification of a sediment, as will be discussed in Chapter 4.

Sequential extraction procedures are designed to access metals in certain phases of the soil or sediment. However, no extractant can specifically target one phase only, without also removing some metals from non-target phases (Förstner 1989, Hall *et al.*, 1996 as cited in Clark *et al.*, 2000). Hence, metals extracted in fractionation studies should be quoted, not by the phase which they are designed to access, but by the extractant used (Clark *et al.*, 2000).

3.3.1 Total Heavy Metal Content

The WNCC sediment contained elevated concentrations of heavy metals (Table 3.2). The concentrations of Cd, Cr, Cu, Pb and Zn exceeded the available guideline values (ICRCL and CLEA) and the sediment is therefore considered to be contaminated and in need of remediation.

3.3.2 pH and Carbonate

Carbonate content was found to be negligible (< 0.01 %) in all samples. Thus, the acid neutralising capacity of the sediment will be low, leaving the sediment vulnerable to acidification. The lack of a carbonate phase also means that those metals extracted by NaOAc cannot be carbonate bound and therefore must be adsorbed.

Oxidation of the WNCC sediment led to significant drying and acidification over time (Table 3.3). Sediment pH at each depth in the sediment core is shown for each time point. The unoxidised (0 day) sediment showed no significant differences in pH with depth. After 4 days of oxidation the

Table 3.2 Total heavy metal content and other general properties of the Woolston New Cut Canal sediment used in the current experiment, n = 6

Heavy Metal	**Al**	**Fe**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Mean (mg kg^{-1})	42976	43517	21.4	917	639	81	1291	3152
SD	4434	1415	2.0	30.3	76.5	9.0	37.8	664

sediment was exhibiting significant differences between depths, with the lowest pH in the surface layer. Even the pH in the deepest (3-x cm) layer was significantly different from the pH in the unoxidised sediment at this time ($p < 0.005$). The surface layer, which was in direct contact with the air, consistently had the lowest pH. The pH in this layer dropped form an initial value of 5.85 to a value of 3.67 after 41 days of oxidation. Over this same period, the pH in the middle (1 – 3 cm layer) dropped to 4.52, and the pH in the bottom (3 – x cm) layer dropped to 4.60. The rapidity of the appearance of significant differences in pH in the sediment during even the shortest periods of oxidation confirms the findings of the low carbonate content and the low acid buffering capacity of this sediment. Although sulphide/sulphate ratios were not measured, the change in pH was almost certainly due to the oxidation of sulphides producing H_2SO_4 (Tack *et al.*, 1997), coupled with the lack of buffering carbonate minerals. The less rapid pH drop in the deeper sediment layers can be explained by the lesser exposure of these levels to oxygen. Due to the lack of structure within the sediment and the small particle size, oxygen diffusion down the profile will be slow, especially whilst the sediment is wet.

The sediment studied by Gambrell *et al.* (1991) had a negligible carbonate content and also underwent significant acidification upon oxidation. The anoxic sediment had a pH of 7 and the oxidised sediment, a pH of 3. However, sediments used by other authors (Tack *et al.*, 1996, Zoumis *et al.*, 2001 Caille *et al.*, 2003) did have significant carbonate contents and showed little or no change in pH during oxidation. It is thus likely that metal fractionation changes in these sediments were more a reflection of changes in redox potential than pH. The decrease in pH seen in the WNCC sediment was not expected or accounted for in the hypothesis and it likely that the decreasing pH will have affected heavy metal behaviour.

3.3.3 Leachate

Leachate was only produced up until 4 days of oxidation. Table 3.4 shows that significant leaching of exchangeable bases (Ca, Mg, K) was occurring during this time. Iron and Al in solution increased by factors of 50 and 137 respectively, between 2 days and 4 days of oxidation. Heavy metals also showed an increase in concentrations in the leachate, suggesting oxidation-induced

Table 3.3 Change in sediment pH and moisture content during the oxidation period. Moisture content was generally not significantly different with depth and results are pooled. Values represent means (SD) (n = 4).

	pH Core Depth (cm)			**Sig.**	**Moisture content %**
Days of Oxidation	**0-1**	**1-3**	**3-x**	**p-value**	**All depths**
0		5.85		1.000	140 (5.5)
4	4.57	5.20	5.44	0.036	130 (4.1)
6	4.03	4.44	5.01	0.024	125 (7.1)
16	4.67	5.04	5.13	0.123	127 (16.0)
20	4.28	4.92	4.94	0.019	123 (14.1)
24	3.96	4.76	5.23	0.048	116 (4.1)
36	4.42	5.32	5.54	0.032	103 (13.2)
41	3.67	4.52	4.60	0.029	93 (7.9)
48					80 (2.3)
Sig. (p-value)	< 0.001	<0.001	0.004		

Table 3.4 Concentrations of elements in the leachate collected from the tanks. Values represent means (SD) (n = 12)

	After 2 days of oxidation	**After 4 days of oxidation**
vol. (mL)	90 (42)	76 (31)
Element	**(mg L^{-1})**	**(mg L^{-1})**
Ca	288 (80)	331 (121)
Mg	48 (10)	61 (28)
K	6.7 (1.4)	10.2 (62)
Fe	1.3 (3.5)	65 (114)
Al	0.15 (0.4)	20.5 (38)
Cd	0 (0)	0.01 (0)
Cr	0 (0)	0.08 (0.2)
Cu	0.03 (0)	0.09 (0.2)
Ni	0.03 (0)	0.09 (0.1)
Pb	0.04 (0)	0.14 (0.2)
Zn	0.11 (0.1)	0.57 (1.3)
S	154 (48)	194 (48)

mobilisation. After deconstruction of the tanks, the sand layer was stained orange with Fe oxide. The sand showed evidence of Fe (0.6 %) and Al (0.3 %) accumulation, and associated scavenging of Cd, Cr, Cu, Ni, Pb and Zn from the leachate (data not shown).

3.3.4 Moisture Content

Moisture content of the sediment (Table 3.3) was only found to be statistically significantly different at different depths in the sediment at 4 days of oxidation (data not shown), when the deeper (3 - x cm) layer of sediment was significantly wetter than the top cm ($p < 0.05$). After this time, loss of water must have been through surface evaporation as leaching ceased. This upward movement of water probably prevented large discrepancies within the profile. Significant sediment drying occurred over the course of the experiment, from the initial moisture content of 140 %, to 80 % of the dry weight of the sediment after 48 days. Field capacity for this sediment was 93 % moisture, a value reached at 41 days of oxidation.

3.3.5 Dithionite

The "0 day" dithionite extracts were acidified and massive precipitation of elements occurred. The samples were misplaced and unfortunately, the precipitate was never digested. As such, results for the "dithionite-extractable" metals at day 0 are best estimates, calculated by summing the metals present in the other fractions and subtracting this value from the mean of the total metal extracted on days 2 to 6.

3.3.6 Iron

The percentage of iron extracted with each extractant (out of total Fe extracted) over the 48-day oxidation period is shown in Figure 3.2 a and c. Figure 3.2 b gives the concentrations (mg kg^{-1}) of iron in the NH4Cl-extractable and NaOAc-extractable fractions of the 0 – 1 cm layer (Fig. 3.2 a). A large proportion of total Fe was extracted by dithionite (Figs. 3.2 a + b), even in the highly reduced, black sediment. Dithionite-extractable Fe is generally quoted as being Fe oxide-Fe. It is highly

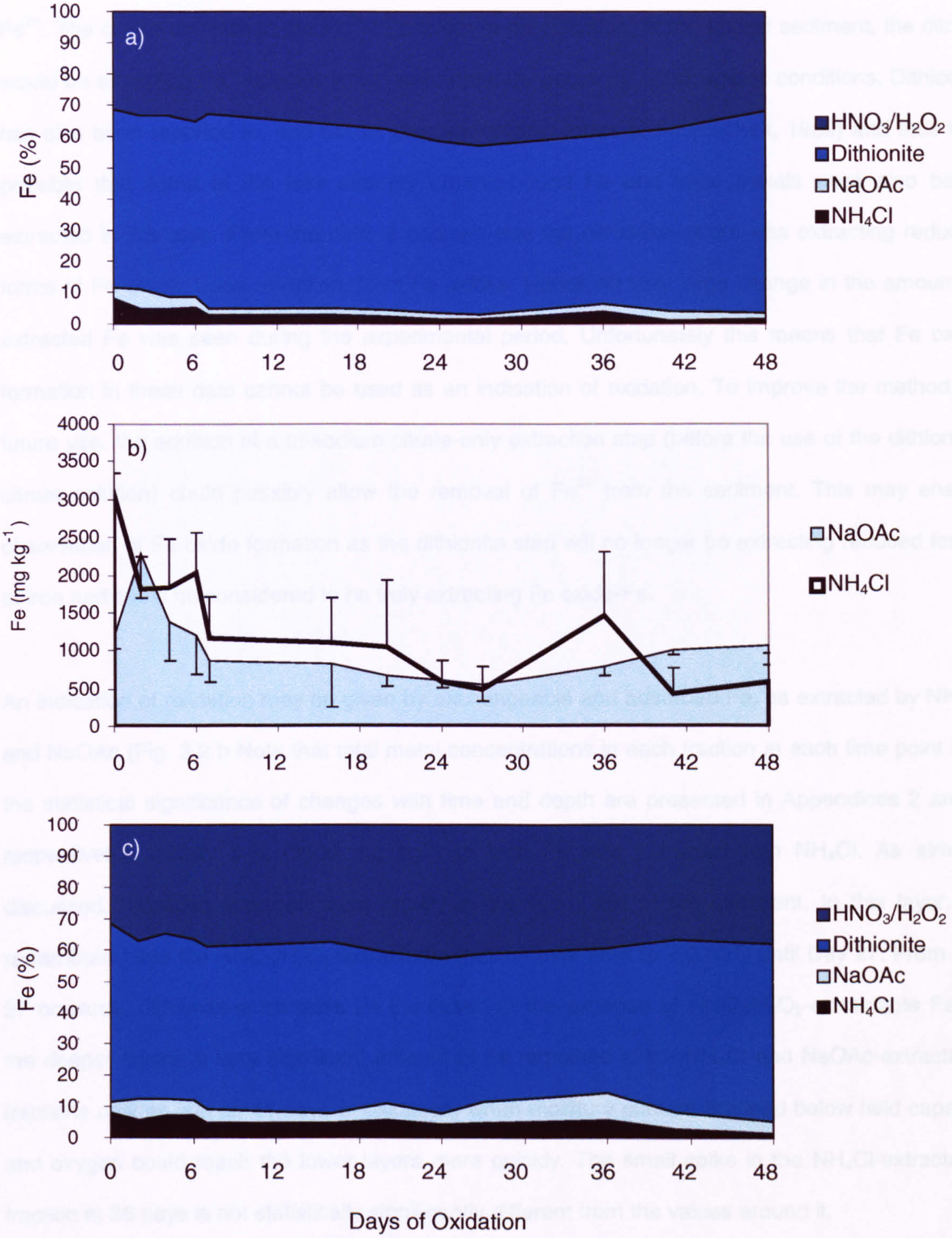


Figure 3.2. **Iron** extracted at each stage of the sequential extraction procedure as a percentage of total Fe extracted. Mean recovery of Fe by sequential extraction was 88% of total. a) 0-1 cm b) 0-1 cm, NH_4Cl and NaOAc extractants only c) 1-3 cm. Values are mean, (n = 4). The error bars on graph b) represent one standard deviation.

unlikely that high concentrations of Fe oxides were present in the reduced sediment. When the dithionite-citrate extractant comes into contact with Fe oxides, the dithionite reduces Fe^{3+} to soluble Fe^{2+}. The citrate complexes the Fe^{2+} to prevent re-precipitation. In the anoxic sediment, the citrate would be extracting Fe^{2+} species which were naturally occurring under anoxic conditions. Dithionite has also been reported to, and not to, dissolve organic forms of Fe (Beckett, 1989) and thus it is possible that some of the less strongly organic-bound Fe and trace metals were also being extracted in this step. From the data, it appears that the dithionite-citrate was extracting reduced forms of Fe which, upon oxidation, form Fe oxides. Hence no very large change in the amount of extracted Fe was seen during the experimental period. Unfortunately this means that Fe oxide formation in these data cannot be used as an indication of oxidation. To improve the method for future use, the addition of a tri-sodium citrate-only extraction step (before the use of the dithionite-citrate solution) could possibly allow the removal of Fe^{2+} from the sediment. This may enable observation of Fe oxide formation as the dithionite step will no longer be extracting reduced forms of iron and could be considered to be truly extracting Fe oxide-Fe.

An indication of oxidation may be given by exchangeable and adsorbed Fe, as extracted by NH_4Cl and NaOAc (Fig. 3.2 b Note that total metal concentrations in each fraction at each time point and the statistical significance of changes with time and depth are presented in Appendices 2 and 3 respectively). Initially 8 % (3600 mg kg^{-1}) of total Fe was extracted with NH_4Cl. As already discussed, oxidation occurred most rapidly in the top 1 cm of the sediment. In this layer, Fe redistributed into the HNO_3/H_2O_2-extractable fraction over time ($p < 0.001$) until Day 27. From day 27 onwards, dithionite-extractable Fe increased at the expense of HNO_3/H_2O_2-extractable Fe. In the deeper layers, a very significant amount of Fe remained in the NH_4Cl and NaOAc-extractable fractions until as late as 41 days of oxidation, when moisture content dropped below field capacity and oxygen could reach the lower layers more quickly. The small spike in the NH_4Cl-extractable fraction at 36 days is not statistically significantly different from the values around it.

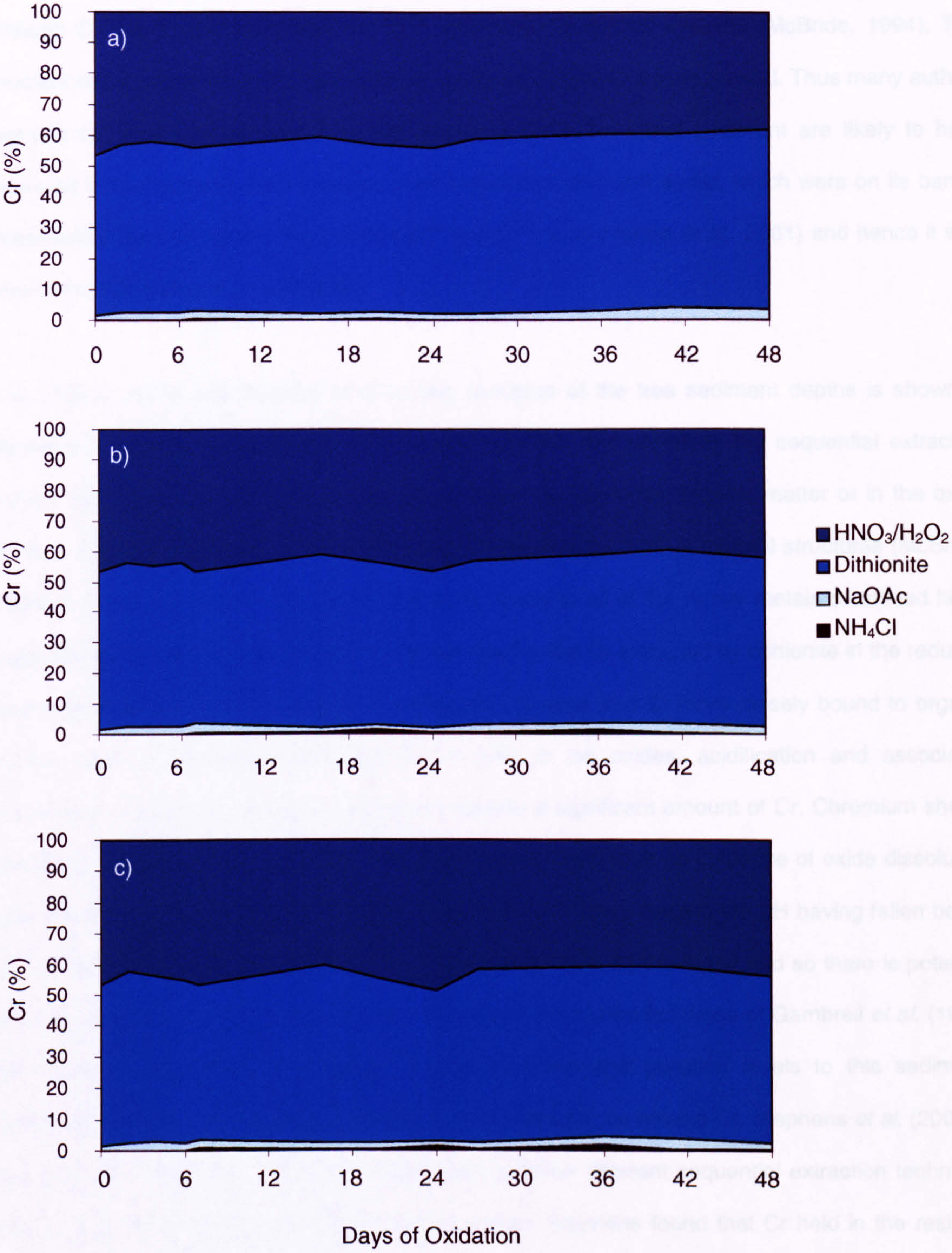


Figure 3.3. **Chromium** extracted at each stage of the sequential extraction procedure as a percentage of total Cr extracted, at 3 depths within the sediment core: a) 0-1 cm b) 1-3 cm c) 3 cm to the core bottom. Mean recovery of Cr by the sequential extraction technique was 87 % of total. Values are mean, (n = 4).

3.3.7 Chromium

Though Cr^{6+} is a very toxic species, Cr^{3+}, dominates in natural systems (McBride, 1994). This trivalent chromium tends to be insoluble and does not constitute a toxic hazard. Thus many authors exclude Cr from their studies. The high levels of Cr in the canal sediment are likely to have occurred from discharge from a tannery and associated chemical works, which were on its banks. Wastewater from tanneries may contain Cr^{3+} and Cr^{6+} salts (Verma *et al.*, 2001) and hence it was important to investigate Cr at this site.

The change in the extractability of Cr during oxidation at the tree sediment depths is shown in Figure 3.3. Despite the high total concentration of Cr in this sediment, the sequential extraction shows that more than 95 % of Cr was bound either by sulphides, organic matter or in the oxide fraction (Fig. 3.3). Chromium has a tendency to substitute for Fe^{3+} in mineral structures (McBride, 1994) and had the largest dithionite-extractable phase of all of the heavy metals examined here, suggesting a close association with Fe. As with the Fe, the Cr extracted by dithionite in the reduced sediment is likely to have consisted of reduced Cr species and Cr more loosely bound to organic matter. Due to the large percentage of Cr held in the oxides, acidification and associated dissolution of this phase has the potential to liberate a significant amount of Cr. Chromium should therefore be regarded as a potential hazard. However, there was no evidence of oxide dissolution after 48 days of oxidation (dithionite-extractable Fe was rising), despite the pH having fallen below 3.7 in the top cm of the soil. Below pH 3.5, dissolution may start to occur and so there is potential for increased future toxicity of Cr. These Cr data seem to contradict those of Gambrell *et al.* (1991) who studied a sediment very similar in characteristics and pollution levels to this sediment. Gambrell reports "no apparent pH or redox potential effects" on soluble Cr. Stephens *et al.* (2001 b) examined Cr fractionation change, though using a rather different sequential extraction technique (the BCR extraction). Over a 12-week drying period, Stephens found that Cr held in the residual (aqua-regia-extractable) phase decreased from around 95 % to just under 90 %. A small increase in Cr was seen in the reducible phase (equivalent to dithionite-extractable) and a larger increase

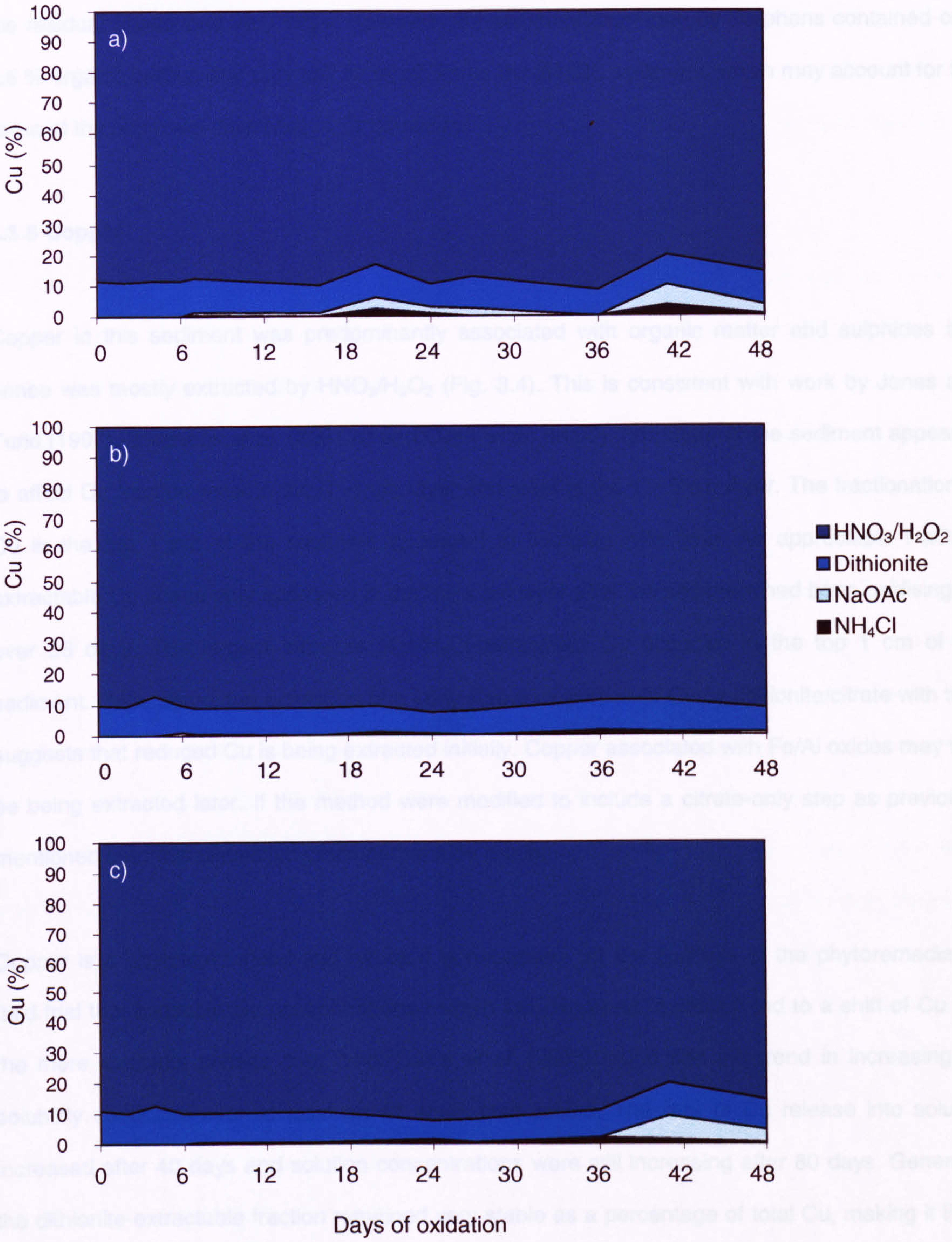


Figure 3.4. **Copper** extracted at each stage of the sequential extraction procedure as a percentage of total Cu extracted, at 3 depths within the sediment core: a) 0-1 cm b) 1-3 cm c) 3 cm to the core bottom. Mean recovery of Cu was 83 % of total. Values are mean, (n = 4).

was seen in the oxidisible phase. This appears to disagree in some respects to the results found in the current study, as the reducible phase in the sediment studied by Stephens was very small and the residual phase was very large. However, the sediment examined by Stephens contained only 2.6 % organic carbon and only half as much Fe as the WNCC sediment, which may account for the some of the observed difference in Cr behaviour.

3.3.8 Copper

Copper in this sediment was predominantly associated with organic matter and sulphides and hence was mostly extracted by HNO_3/H_2O_2 (Fig. 3.4). This is consistent with work by Jones and Turki (1997), Stephens *et al.* (2001 b) and Clark *et al.* (2000). Oxidation of the sediment appeared to affect Cu fraction most in the 0 –1 cm layer and least in the 1 – 3 cm layer. The fractionation of Cu in the top 1 cm of the sediment appeared to fluctuate with time. An appreciable NaOAc-extractable Cu phase only appeared in the 3 – x cm layer after the sediment had been oxidising for over 36 days. The largest increase in NH_4Cl-extractable Cu occurred in the top 1 cm of the sediment. Once again, the extraction of a fairly stable proportion of Cu by dithionite/citrate with time suggests that reduced Cu is being extracted initially. Copper associated with Fe/Al oxides may well be being extracted later. If the method were modified to include a citrate-only step as previously mentioned then this distinction could perhaps be made.

Copper is a phytotoxic metal and hence it is necessary for the success of the phytoremediation field trial that available Cu concentrations remain low. However, oxidation led to a shift of Cu into the more available phases over time. Caille *et al.* (2003) found that the trend in increasing Cu solubility continued over at least an 11 week time period. The rate of Cu release into solution increased after 40 days and solution concentrations were still increasing after 80 days. Generally, the dithionite-extractable fraction remained very stable as a percentage of total Cu, making it likely that the NH_4Cl-extractable Cu was coming directly from the oxidation of sulphides and oxic breakdown of organic matter. Copper is strongly complexed by organic matter, more strongly than any other transition metal, but it is also a chalcophile, meaning that it has a tendency to form very insoluble sulphide minerals (McBride, 1994). Oxidation will result in release of the majority of sulphide-bound Cu, due to the disappearance of the sulphide phase, and also a certain proportion of organic-bound Cu. Exchangeable Cu can be expected to increase for as long as sulphide oxidation is occurring. The NaOAc-extractable Cu fraction started to appear after the NH_4Cl-extractable Cu and it is possible that the newly liberated Cu was leaving exchange sites and becoming adsorbed onto surfaces of Fe oxides and other cation binding sites, newly-created during oxidation.

The last leachate was collected from the tanks after 4 days of oxidation. In the later stages of the experiment, the bottom of the sediment was therefore lying on a dry sand and gravel layer. The data showing a larger increase in the adsorbed phase of Cu in the 3-x to the 1-3 cm depth, is then evidence that after 36 days, significant oxidation was occurring from both the bottom and the top of the sediment (see also Fig. 3.6 and Table 3.3).

Guo *et al.* (1997) reduced an oxidised bottom sediment and found that, initially, as E_h fell, more soluble Cu was liberated. However, as the E_h dropped below 0 mV, soluble Cu quickly decreased by a factor of 10. Gambrell *et al.* (1991) also found that soluble Cu tended to increase with increasing redox potential, though not significantly. If the WNCC sediment followed a similar trend to the sediment examined by Guo, then NH_4Cl-extractable Cu could increase by a factor of 10 when the sediment becomes more oxidised, and thus pose a significant risk. The natural acidification of this sediment will also increase solution concentrations of Cu, as binding sites such as Fe/Al hydroxides and clay surfaces have a pH dependant charge, which will tend to be more positive at low pH. A longer-term study of Cu fractionation change during oxidation is therefore necessary to better characterise the potential risk from this metal. However, if availability continued to increase with increasing oxidation then toxicity issues may arise.

3.3.9 Lead

Though Pb is thought of as the least mobile heavy metal in soil (McBride, 1994), in the oxidising WNCC sediment this appeared not to be the case (Fig. 3.5). From the outset there was a significant pool of adsorbed Pb at all depths within the sediment. This increased in the first 2 days

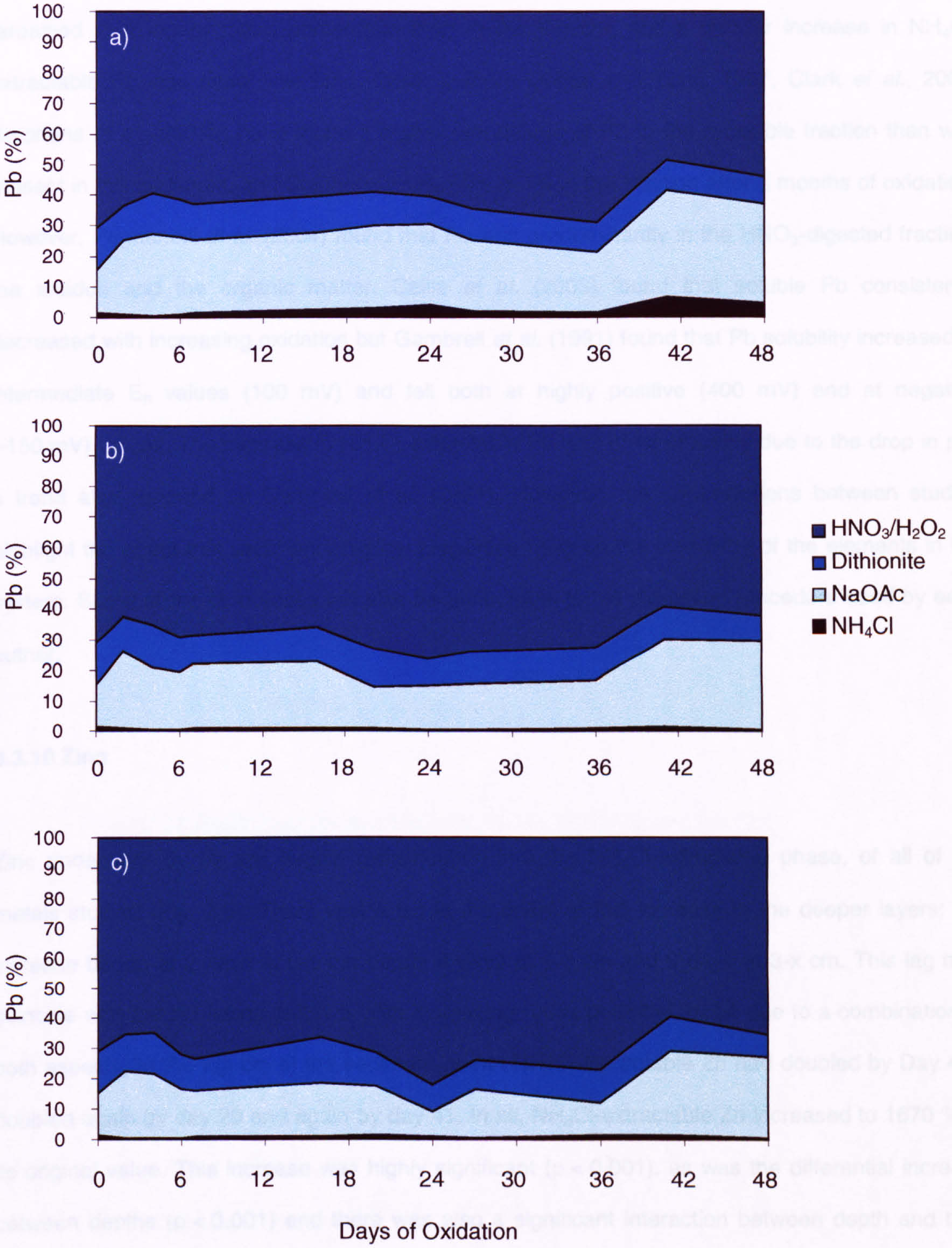


Figure 3.5. **Lead** extracted at each stage of the sequential extraction procedure as a percentage of total Pb extracted, at 3 depths within the sediment core: a) 0-1 cm b) 1-3 cm c) 3 cm to the core bottom. Mean recovery of lead was 83 % of total. Values are mean, (n = 4).

of oxidation ($p < 0.001$). In the top cm, the adsorbed Pb then remained fairly stable ($p = 0.772$), but the NH_4Cl-extractable Pb increased ($p < 0.01$). In the deeper layers, HNO_3/H_2O_2-extractable Pb remained at a higher mean percentage than in the top cm, and a smaller increase in NH_4Cl-extractable Pb was seen over time. Other authors (Jones and Turki, 1997, Clark *et al.*, 2000, Stephens *et al.*, 2001b) have found a higher percentage of Pb in the reducible fraction than was present in this sediment, and Stephens found 80% of Pb in this fraction after 3 months of oxidation. However, Pagnanelli *et al.* (2004) found that Pb was predominantly in the HNO_3-digested fraction, the residue and the organic matter. Caille *et al.* (2003) found that soluble Pb consistently decreased with increasing oxidation but Gambrell *et al.* (1991) found that Pb solubility increased at intermediate E_h values (100 mV) and fell both at highly positive (400 mV) and at negative (-150 mV) values. The increase in NH_4Cl-extractable Pb was most probably due to the drop in pH, a trend also reported by Gambrell *et al.* (1991). However, the contradictions between studies highlight the effect that sediment physical properties have on the chemistry of the elements in the system. Some of the differences will also be attributable to the extraction procedure used by each author.

3.3.10 Zinc

Zinc underwent by far the largest redistribution into the NH_4Cl-extractable phase, of all of the metals studied (Fig. 3.6). There was a lag in the onset of this increase in the deeper layers: the increase began at 2 days at 0-1 cm depth, 4 days at 1-3 cm and 6 days at 3-x cm. This lag may coincide with the pH falling below 5, with increasing redox potential, or be due to a combination of both aspects. In the top cm of the sediment, mean NH_4Cl-extractable Zn had doubled by Day 4. It doubled again by day 20 and again by day 41. In all, NH_4Cl-extractable Zn increased to 1670 % of its original value. This increase was highly significant ($p < 0.001$), as was the differential increase between depths ($p < 0.001$) and there was also a significant interaction between depth and time ($p < 0.001$). Gambrell *et al.* (1991) found that at pH 5, soluble Zn increased from 0.8 mg kg^{-1} to 415 mg kg^{-1} as redox potential increased from 100 to 400 mV, illustrating the significant effect which oxidation has on Zn speciation. Gambrell also found that increasing the pH to 6.5 reduced soluble Zn to 34 mg kg^{-1} at 400 mV. The same effects were evident in this sediment. The effect of

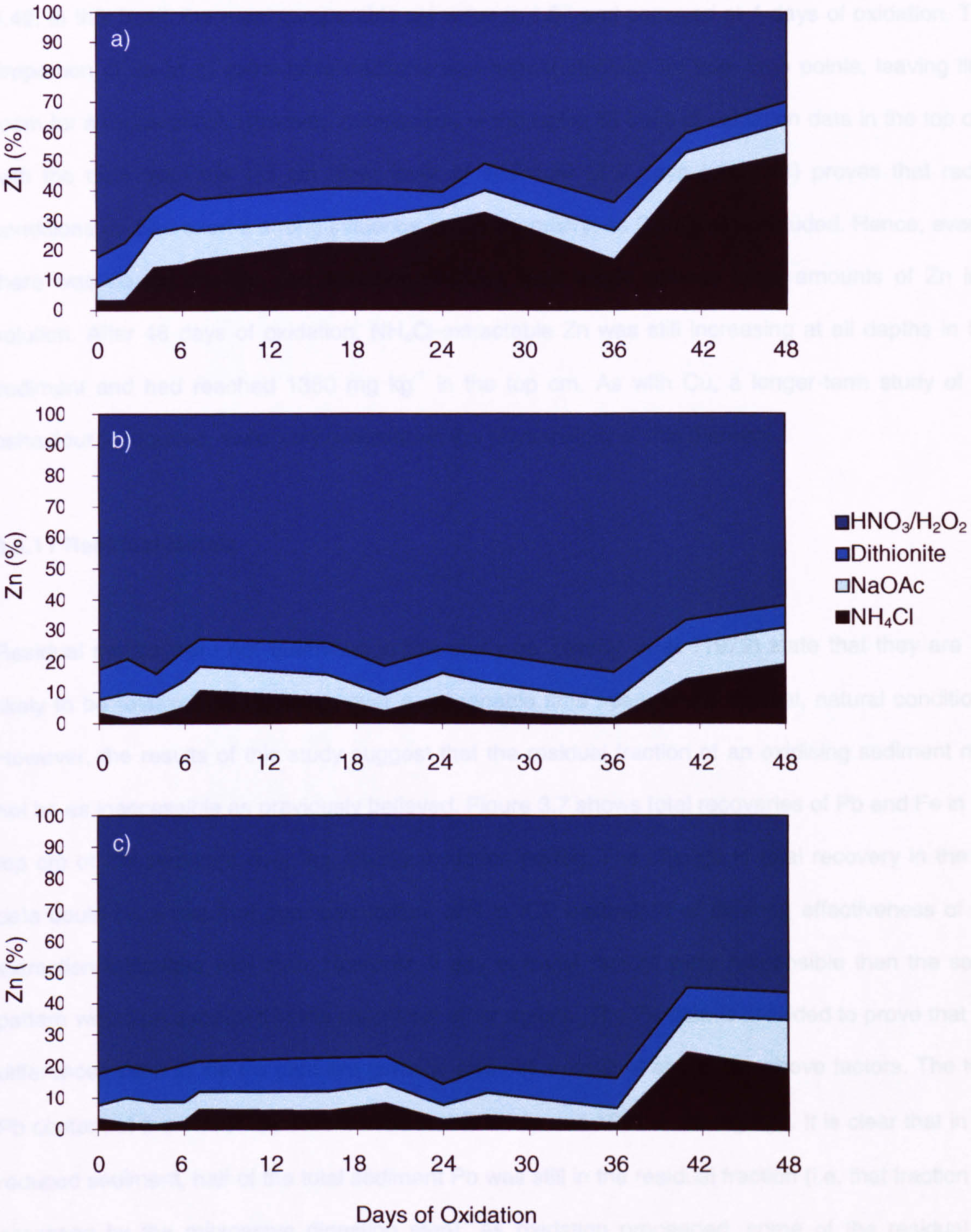


Figure 3.6. **Zinc** extracted at each stage of the sequential extraction procedure as a percentage of total Zn extracted, at 3 depths within the sediment core: a) 0-1 cm b) 1-3 cm c) 3 cm to the core bottom. Mean recovery of total Zn was 74 %. Values are mean, (n = 4).

pH on NH_4Cl-extractable Zn is visible in the 0-1 cm layer of the sediment by comparing the data of 4 and 36 days of oxidation. In the top cm of the sediment at 36 days of oxidation, the pH rose to 4.42. In this layer, the most comparable pH value is 4.57 and occurred at 4 days of oxidation. The proportion of Zn in all extractable fractions was almost identical for both time points, leaving little room for a redox effect. However, comparison of the same 36 days of oxidation data in the top cm, with the data from the 1-3 cm deep layer of sediment at 6 days (pH 4.44) proves that redox conditions do also exert a strong influence on Zn chemistry, as Gambrell concluded. Hence, even if there was no pH change, the oxidation process itself would release large amounts of Zn into solution. After 48 days of oxidation, NH_4Cl-extractable Zn was still increasing at all depths in the sediment and had reached 1360 mg kg^{-1} in the top cm. As with Cu, a longer-term study of Zn behaviour is required, especially considering the phytotoxicity of this element.

3.3.11 Residual Metals

Residual metals were not quantified in this study as Tessier *et al.* (1979) state that they are not likely to be released into solution over a reasonable time span, under normal, natural conditions. However, the results of this study suggest that the residual fraction of an oxidising sediment may not be as inaccessible as previously believed. Figure 3.7 shows total recoveries of Pb and Fe in the top cm of the sediment over the 48-day oxidation period. The change in total recovery in the Pb data could be a result of poor calculation, drift in ICP calibration or differing effectiveness of the extraction technique with time. However, if any of these factors were responsible then the same pattern would be expected in the results for other metals. The Fe data is provided to prove that the differences seen in the Pb data are genuine and not a result of any of the above factors. The total Pb content of the WNCC sediment used in this study was 1291 ± 38 mg kg^{-1}. It is clear that in the reduced sediment, half of the total sediment Pb was still in the residual fraction (i.e. that fraction not accessed by the microwave digestion step). As oxidation proceeded, some of the residual Pb redistributed into the HNO_3/H_2O_2-extractable fraction. This trend was also seen in the Pb 1-3 cm fraction, and to a smaller extent in the 3-x cm fraction (data not shown). Copper and Cr showed a similar pattern, though less pronounced. The nature of the mobilisible residual phase is unknown. The use of the H_2O_2 in the digestion step left the sediment bleached of organic matter, therefore it

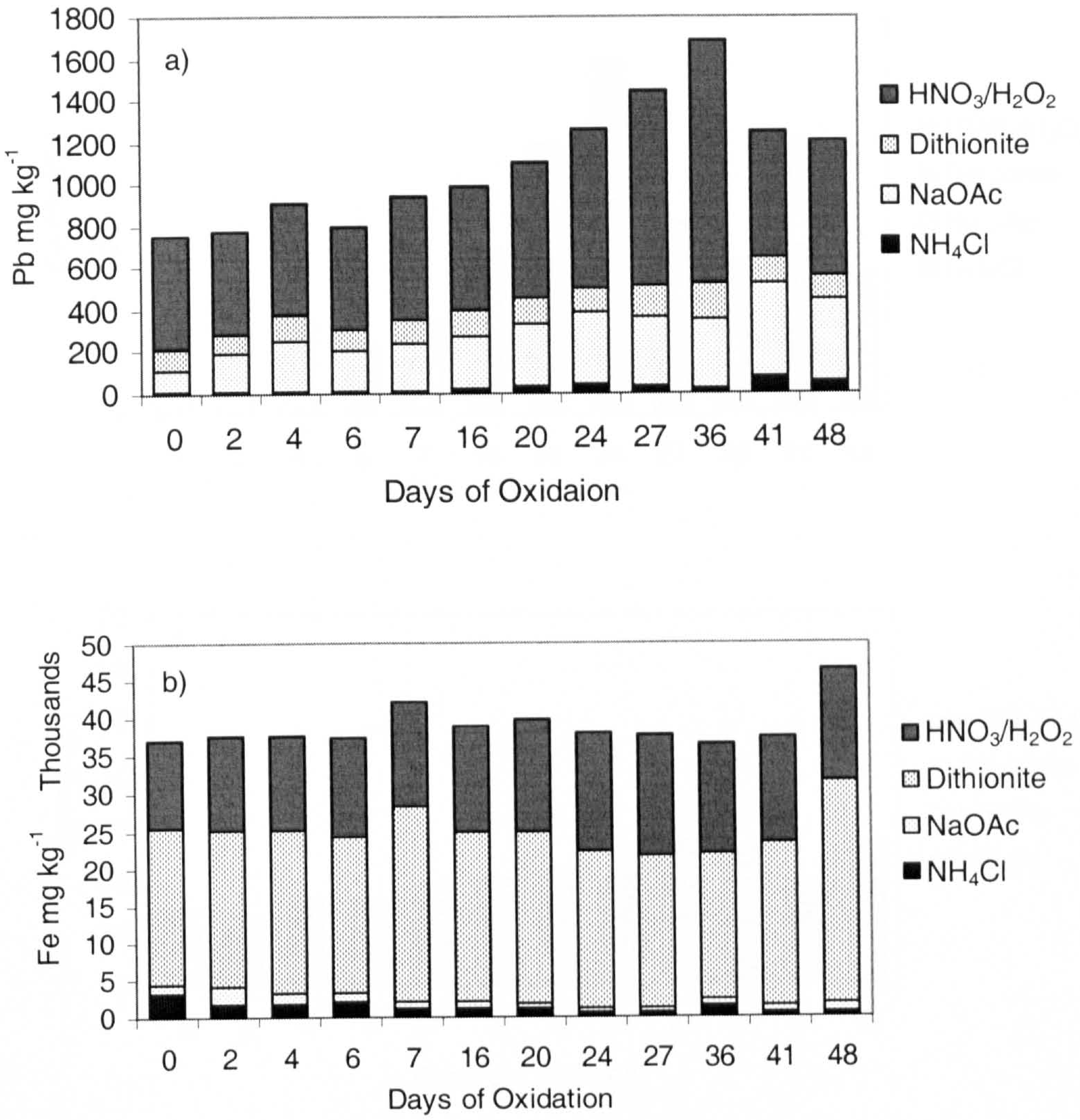


Figure 3.7. Change in the sum of all metal fractions extracted over time from the top 1 cm of the soil core for a) Lead and b) Iron. Values represent means (n = 4).

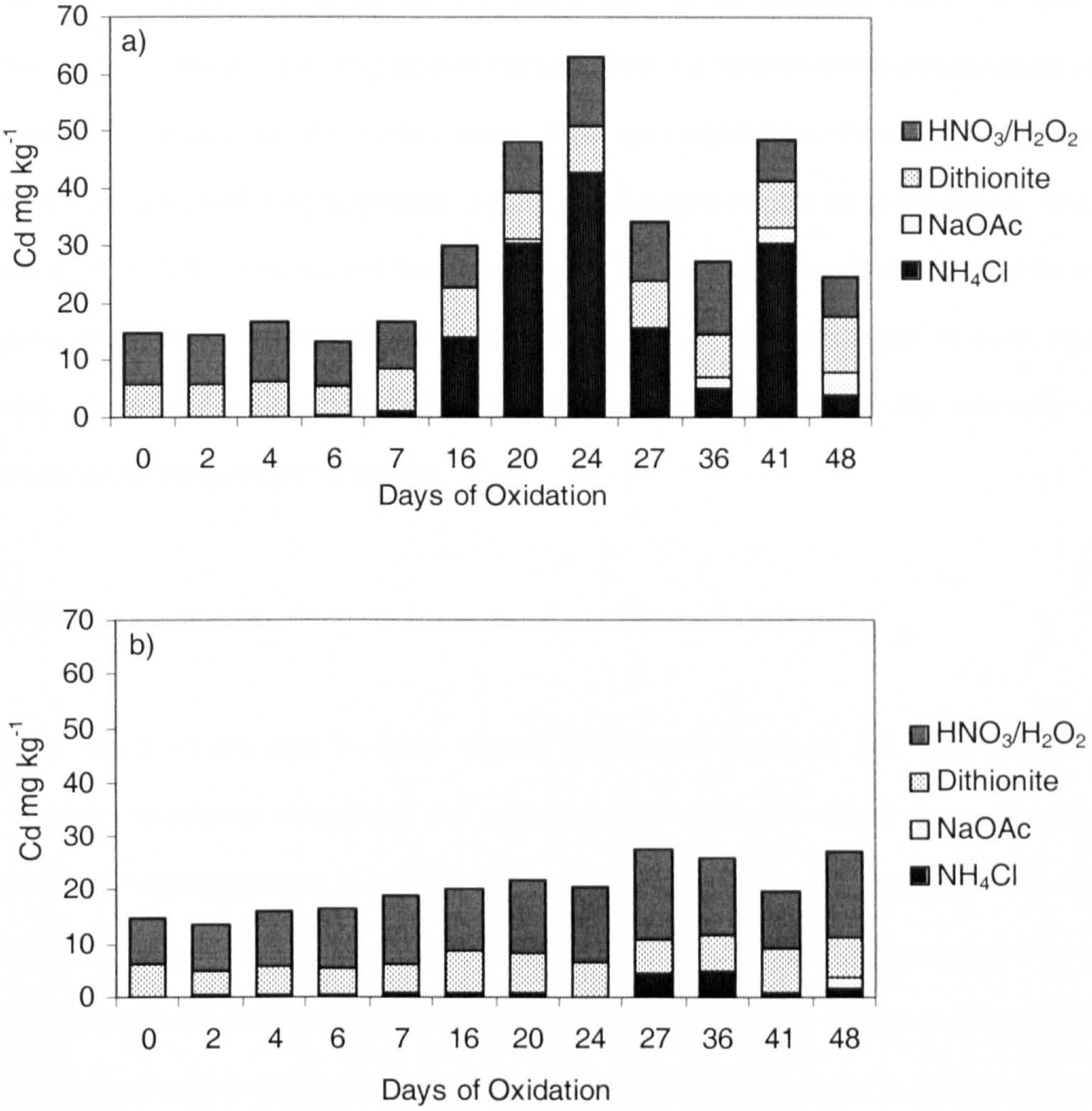


Figure 3.8. Cadmium extracted from the sediment during the sequential extraction at a) 0-1 cm, and b) 1-3 cm depths in the sediment core. Values represent means (n = 4).

is highly unlikely to have been organic in nature. A possible explanation, is that a phase existed which became extractable during the natural process of oxidation, acidification and short-term weathering, but not during digestion with a strong oxidising acid and a strong oxidising agent. Perhaps a sediment component acted as a catalyst for the mobilisation reactions, but was destroyed upon the addition of the strong acid or the peroxide. To determine the exact nature of the potentially-mobilisible residual phase, further study must be undertaken. However, it is possible that the phase constitutes resistant sulphides, as Cd, Cr, Cu and Pb are all chalcophilic. The Pb, Cu and Cr data (Figs. 3.3 - 3.5) should be viewed with this increase in total-extracted in mind. Though proportions of these metals remained the same, actual concentrations in each fraction were increasing with time, until the "total" concentration was reached. The 48-day recovery of Pb by the sequential extraction was 97 % of total.

3.3.12 Cadmium

The Cd data for the 0 – 1 cm and 1 – 3 cm depths are shown in Figure 3.8. Due to the large changes in total Cd recovered throughout the experiment in the top 1 cm of the sediment, the results have not been represented in the same form as the other metals. The data for the 1 – 3 cm layer are within what would be expected, with an NH_4Cl-extractable phase appearing over time. Some NH_4Cl-extractable Cd then redistributed into the NaOAc-extractable phase at 48 days, in line with the original hypothesis. In the top 1 cm however, the Cd concentrations in the NH_4Cl-extract varied considerably between replicates and time points. Total Cd in this sediment was 21.4 ± 2 mg kg^{-1} – a value exceeded by up to two times in the NH_4Cl-extractable phase alone. Disregarding this phase for now, in the top 1 cm of the sediment Cd was initially present in the HNO_3/H_2O_2-extractable and dithionite/citrate-extractable phases only. After the appearance of the NH_4Cl-extractable phase, an NaOAc-extractable phase began to appear and increased in size over time. As hypothesised, this is probably due to the redistribution of NH_4Cl-extractable Cd in to less available phases through equilibrium processes.

The NH_4Cl-extractable Cd in the top 1 cm of the sediment reached very high concentrations as previously noted. The standard deviations of these data are large as concentrations were not elevated in all samples. Cadmium is one of the most mobile heavy metals in the WNCC sediment. Due to the method of drying which occurred in the tanks (mostly evaporative), it is possible that soluble Cd moved from the deeper sediment layers to the surface layer with the sediment moisture flow. Thus, metal concentrations in the top 1 cm of the sediment became elevated with respect to other layers. The apparent scale of the elevation would be increased due to the smaller sediment volume in the top 1 cm of the sediment, compared to the volume of sediment below it. The large standard deviation of the data and the decrease in the concentrations of NH_4Cl-extractable Cd after 24 days of oxidation suggest that the Cd may be being liberated from localised clusters of Cd-bearing sulphides, either at, or below the surface. Lateral diffusion of the soluble Cd would then lead to the decrease in NH_4Cl-extractable Cd seen after 24 days.

The large release of soluble Cd was an effect of oxidation of the sediment, as shown by the initial lack of this phase, and by the 1-3 cm depth data. As Cd is chalcophilic, the oxidation of Cd-bearing sulphides is the obvious source of this Cd. Stephens *et al.* (2001 b) found that oxidation of their sediment caused major immobilisation of Cd, though they comment that this is in disagreement with other studies, which also describe release of Cd from sulphides.

3.4 Conclusions

It was hypothesised that the sediment heavy metals would be released into more soluble forms during oxidation and would then re-distribute into less-available phases. Oxidation did lead to an increase in the NH_4Cl-extractable phases of most heavy metals studied but the expected redistribution did not occur. This was because of the acidification of the sediment during oxidation, due to the lack of a buffering carbonate mineral phase. The low pH of the sediment favoured the heavy metals staying in more available forms. This was particularly obvious for Zn and Cd, the high NH_4Cl-extractable concentrations of which would be a pollution concern in an environmental system. Due to this oxidation-induced acidification, it was not possible in this experiment to differentiate between the effect of oxidation on metal behaviour and the effect of pH on metal behaviour. The least mobile metal was Cr and it was not considered to pose a toxic hazard unless dissolution of Fe oxides occurred. The chalcophilic metals exhibited redistribution from the residual phase into extractable phases during oxidation, and may have predominantly entered the HNO_3/H_2O_2-extractable phase e.g. Pb. The residual phase that liberated these metals is not known and further investigation is required, though resistant sulphides are a possibility.

Monitoring of Cd, Cu, Pb and Zn over a longer time period would be beneficial as the NH_4Cl-extractable phases are a pollution concern. Perhaps the most serious potential toxicity problems were those posed by Zn and Cd availability, and low pH. Due to its phytotoxicity and increasing availability, Zn is the metal most likely to lead to failure of the phytoremediation field trial. The increase in Cu availability would compound the toxicity of the sediment if concentrations continued to increase in the more available fractions over time. During the early stages of oxidation, the top 1 cm of the sediment presented the greatest toxic risk. As time goes on, the sediment would oxidise to greater depths and it is likely that the sediment at the field site would oxidise in a similar manner. Thus, over time, high tree mortality could be expected if nothing was done to counteract the acidity.

Generally, oxidation of the sediment led to development of a low pH, and increased the availability of toxic heavy metals. If the sediment were dredged to the side of the canal, or deposited onto land, then rainfall may lead to the production of more leachate than was seen in this study. Leachate and run-off therefore have a high probability of containing high concentrations of heavy metals, which may pollute the surrounding canal. Leachate would also be expected to have a low pH which would also have a detrimental effect on the surrounding canal. Therefore, dredging of the sediment converted a relatively inert, stable sediment into a toxic hazard and such activity would not be advised on a large scale without the addition of amendments such as lime.

CHAPTER 4

Changes in Metal Lability During Oxidation of a Contaminated Canal Sediment

4.1 Introduction

The results presented in Chapter 3 showed that metal fractionation changed throughout a 48-day oxidation period. The measurement of fractionation is operationally defined as it is dependant upon the extractants used and the sequence in which they are used. Experimental work presented in the present chapter investigates the change in metal lability during oxidation using isotope dilution techniques. These techniques do not require harsh chemical methods and thus the sediment undergoes much less physical and chemical alteration during the experimental process. Oxidation of the sediment was through a "slow" oxidation procedure – in air over a 48-day period – and via a "rapid" procedure – by the addition of the oxidising agent hydrogen peroxide. The peroxide oxidation procedure was tested to determine its viability as a method for accurately and quickly predicting metal lability change with changing pH (and E_h) within the sediment.

4.1.1 Assessment of Heavy Metal Availability

Assessment of changes in metal availability has typically been through the use of techniques such as sequential extraction schemes (Guo *et al.*, 1997, Stephens *et al.*, 2001 a, Clark *et al.*, 2000, Stephens *et al.*, 2001 b, van Ryssen *et al.*, 1999, Tack *et al.*, 1996, Kersten and Förstner, 1989), leaching studies (Stephens *et al.*, 2001 b), and solubility studies (Caille *et al.*, 2003). Sequential extractions of reduced sediments show that as the sediment oxidises, metals generally move from the less available fractions (described as "residual") into the more available fractions, which are usually described as "carbonate-bound or adsorbed", "exchangeable" and "Fe/Mn oxide-bound". For example, Zn has been found to redistribute from the "residual" phase, probably present as sulphides and organic matter, into other, more available phases such as Fe/Mn oxide-bound (van Ryssen *et al.*, 1999, Tack *et al.*, 1996) and the carbonate-bound/adsorbed fraction (Zoumis *et al.*, 2001, Caille *et al.*, 2003). Nickel has been reported to be present in the carbonate/adsorbed and residual fractions when a sediment is reduced and during oxidation it either redistributes into the Fe/Mn (oxyhydr)oxide-bound fraction, or remains in the carbonate-bound/adsorbed fraction (Guo *et al.*, 1997, Stephens *et al.*, 2001 a, van Ryssen *et al.*, 1999, Tack *et al.*, 1996).

However, sequential extractions extract an operationally-defined pool of metals and leaching and solubility studies fail to include exchangeable metals adsorbed on the solid phases. In this study, changes in Cd, Cu, Ni and Zn lability (isotopic exchangeability) were measured using isotope dilution techniques (E values), which take into account both solution concentrations and exchangeable metals on the sediment solid phases. E values have been used to study metal lability in soils by a number of authors (Echevarria *et al.*, 1998, Young *et al.*, 2000, Hamon *et al.*, 2002 a, Lombi *et al.*, 2003, Tye *et al.*, 2003) but the author knows of no studies to date examining an oxidation series of contaminated sediments.

4.1.2 E values

The E value technique is used to estimate lability of metals in a soil or sediment. Only metals which have an appropriate isotope i.e. short half-life, safe to work with, or an appropriate stable isotope, can be used at present. When radioactive isotopes are used, a spike of a radioactive isotope of the metal, with known activity, is added to a soil/sediment suspension and left to equilibrate for a given period. The essential assumption of the process is that during equilibration, the isotope acts as the native metal would do and partitions between soil components in the same proportions as the native metal does (Hamon *et al.*, 2002 b). After the equilibration period, the activity in solution is measured and corrected for decay, enabling calculation of the proportion of added isotope, remaining in solution. This in turn gives the proportion of the metal that has exchanged with the labile phases of the soil. Once this proportion is known, and using the above assumption, the total labile metal in the soil can be calculated from the solution concentration of the native metal. However, the general E value technique may overestimate true metal lability due to the presence of colloids in soil and sediment systems. A fraction of the metals incorporated into these colloids may be non-isotopically exchangeable (Lombi *et al.*, 2003) but will still be incorporated in the E value.

This overestimation of lability can be avoided by the addition of a resin step (Lombi *et al.*, 2003), giving the E_r value.

4.1.3 E_r Values

The E_r value is perhaps a better measure of lability than the E value. It entails the use of an ion exchange resin which is added to the supernatant which was used in calculation of the E value. The exchange resin sorbs metals onto its surfaces in the same proportions at which they are found in the supernatant. However, metals occluded in colloids will not exchange and will therefore not be counted in the final measurement of solution concentrations. Extraction of the resin with acid gives the true proportion of isotope to native metal and this is used to calculate the E_r value. A comparison of the E and E_r values via the E/E_r ratio gives a measure of the metals occluded in these colloids. This is important in terms of the biogeochemistry of the metals, as colloids can act as vectors for accelerated contaminant movement (Flury *et al.*, 2002).

Here, E_r values were used to observe changes in metal lability during oxidation in air over a 48-day period (hereafter referred to as "slow" oxidation), and to compare these results with those obtained using hydrogen peroxide as the oxidising agent (hereafter referred to as "rapid" or "peroxide" oxidation).

4.1.4 Aims

- To determine the change in metal lability with increasing oxidation both in air and via oxidation with hydrogen peroxide.
- To establish whether the rapid oxidation process accurately reflected changes in metal lability during the slow oxidation process.
- To compare the lability changes with the fractionation changes reported in Chapter 3.

4.1.5 Hypothesis

The lability of Cd, Cu, Ni and Zn will increase during oxidation. It is probable that the increase in lability will be above that reported in the increase of the NH_4Cl-extractable fraction quantified in Chapter 3 as labile metals may not necessarily have been removed by this extractant. The proportion of total Zn and Cd which become labile are expected to be higher than the proportions of total Cu and Ni which become labile. Using hydrogen peroxide as the oxidising agent is likely to lead to the breakdown of organic matter and thus Cu lability may be higher by this method than by oxidation in air. Whether or not the peroxide oxidation method will prove to be a useful prediction tool uncertain.

4.2 Materials and Methods

4.2.1 Sediment

The sediment used in this experiment was dredged from a disused canal site in Warrington, North West England, currently the location of a phytoremediation field trial as described by Royle *et al.* (2003). The sediment was stored in polyethylene barrels covered with a layer of native water (as described in Chapter 3). For subsampling for the current experiment, the sediment was thoroughly homogenised and a 3 L subsample was taken. This material was sent by courier to CSIRO Land and Water in Adelaide, Australia where the current experiment was conducted. At CSIRO Land and Water, the sediment was recombined and stored in a polyethylene tub in a cold room at 4 ºC until needed.

4.2.2 Slow Oxidation Experiment

Forty-eight days before equilibration of the sediment/water suspension was to begin, approximately 70 g wet sediment (moisture content 162 %) was weighed into a polyethylene container at a layer thickness of 1 cm. The lid was loosely fitted to allow oxidation, whilst limiting evaporation. This process was repeated at 12 other consecutive time points (Table 4.1). The containers were kept in the dark, at 20 °C throughout the experiment.

On the final day of oxidation, a sub-sample was taken from each container and also from the "0 day" sample. Moisture content was determined by drying the sample at 105 °C for 24 h. To prepare the samples for addition of the radioisotopes, duplicate 2 g dry-weight equivalent sediment subsamples from each container was weighed into 50 mL centrifuge tubes and 20 mL deionised water was added to prevent further exposure to the air. This was done 3 times – for Zn and Cd (combined), Cu, and Ni E value determinations. Two drops of toluene were added to each tube to eliminate microbial activity, after which the headspace of each tube was purged with oxygen-free nitrogen gas. The tubes were shaken on an end-over-end shaker for 3 d to allow equilibrium to be reached between the metals in solution and those on the solid phases of the sediment (Hamon *et al.,* 2002 a, Lombi *et al.*, 2003).

4.2.3 Rapid Oxidation

4.2.3.1 Redox Titration

Prior to the start of the experiment, a redox titration curve was obtained using a sub-sample of the sediment. A 10:1 ratio of water to sediment was stirred into a constant suspension with a magnetic stirrer, ensuring that no vortex was present on the surface of the water. A pH meter and an E_h probe were inserted into the suspension to monitor oxidation. Ten µL of 30 % hydrogen peroxide solution (pH 2.9) was added to the suspension, which was monitored until the pH and Eh readings were stable, at which point another 10 µL of hydrogen peroxide was added. This process was continued until the pH remained stable, even after addition of relatively large volumes (100 µL) of hydrogen peroxide. The resulting oxidation curve was used to determine peroxide additions for the rapid oxidation experiment. The end point of the titration was 24 µL g^{-1} after 1 h equilibration time. A range of addition volumes were chosen between 5 and 40 µL of peroxide per g, and then a further 4 additions of 125, 300, 500 and 1000 µL g^{-1} were used to ensure that total oxidation was achieved and to allow for potential buffering processes during the longer experimental equilibration period.

Table 4.1. The change in pH during "slow" oxidation in air, and "rapid" oxidation using peroxide addition. Data are for the Zn and Cd jointly spiked samples. Any differences in addition between metal treatments are indicated with by a *, (n = 2).

Slow Oxidation Experiment		Rapid Oxidation Experiment	
Oxidation Period (days)	pH	Peroxide Addition ($\mu L\ g^{-1}$)	pH
0	6.29	0	6.02
1	5.63	5.0*	5.87
6	5.73	7.5	5.94
8	5.66	12.5*	5.80
13	5.69	15.0	5.75
15	5.52	17.5*	5.70
17	4.78	22.5	5.52
21	5.04	25.0*	5.46
24	5.73	32.5	5.43
27	4.89	35.0*	5.30
30	5.15	40.0***	5.15
34	4.96	125	4.51
41	4.25	300**	3.97
48	4.25	500	3.84
		1000	3.20

* Only in the case of Zn and Cd, this addition rate was not used for Ni.

** 325 $\mu L\ g^{-1}$ used for Ni.

* For Cu 50 $\mu L\ g^{-1}$ peroxide was used. The next 3 additions were not used for Cu. The final addition was 1000 $\mu L\ g^{-1}$.

4.2.3.2 Rapid Oxidation Experimental Procedure

For E value determinations, 2 g dry weight equivalent of the original wet sediment was weighed into 50 mL centrifuge tubes and water (20 mL) and toluene (2 drops) were added. Duplicate samples were weighed out for Zn and Cd, Cu, and Ni E value determinations. The tube was swirled until the sediment was in suspension, and then the required volume of hydrogen peroxide was added. Tube headspaces were purged with oxygen-free nitrogen gas and they were then shaken on an end-over-end shaker for 4 d to allow complete reaction and equilibration. Those with larger additions of peroxide (≥ 125 µL g^{-1}) were left to react until the formation of oxygen bubbles had ceased (to avoid pressure build up inside the tubes) before shaking. Due to the availability of sediment and isotopes, the complete set of peroxide additions (as shown in Table 4.1 as the ^{65}Zn/^{109}Cd spiked sample data) was not carried out for all isotopes. Those samples spiked with ^{63}Ni received peroxide additions as shown in Table 4.1 with the exception of those addition rates marked with a single asterisk. The 300 µL g^{-1} addition of peroxide was also different for the ^{63}Ni spiked samples, being 325 µL g^{-1}. Those samples spiked with ^{64}Cu received all peroxide addition up to 35 µL g^{-1}. The next addition rate after this was then 50 µL g^{-1} and the only other addition rate received by ^{64}Cu spiked samples was 1000 µL g^{-1}.

4.2.4 Isotopic Dilution Techniques

All spiking with radioisotopes was carried out simultaneously using the method of Hamon *et al.* (2002 a) and Lombi *et al.* (2003). After the equilibration period, the pH of the 10:1 water-to-sediment suspension was measured. The samples were then spiked separately with 50 µL of a solution containing either ^{63}Ni (1884 kBq mL^{-1}), ^{64}Cu (60 MBq mL^{-1}) or a mixture containing ^{65}Zn (1000 kBq mL^{-1}) and ^{109}Cd (400 kBq mL^{-1}). Headspaces were repurged with N_2 gas, and the tubes were shaken for a further 3 d to allow equilibration between the radioisotope metal and the native metal, except in the case of the ^{64}Cu spiked samples. Due to the high activity of these samples, spiking was carried out slightly differently. During addition of the ^{64}Cu spike, the lid of the tube was lifted just enough to allow the pipette tip to be inserted. The spike was added as quickly as possible and the lid was immediately replaced, so as to avoid as far as possible, the introduction of oxygen into the tubes. This was done to eliminate the need to repurge the headspace and hence to minimise exposure to the high level of activity in these samples. Also, due to the short half-life (12 h) and the low efficiency of the gamma counting for the ^{64}Cu isotope, the samples were shaken for 1 d only.

At the end of the shaking period, an aliquot of each suspension was taken for the determination of pH. The remaining suspension was centrifuged at 3000 *g* for 30 minutes. The supernatant was filtered through a 0.2 µm Sartorius filter and the solids were discarded. The ^{63}Ni activity was determined using a LKB Wallac 1215 Rackbeta II Liquid Scintillation Counter. Two mL of the supernatant were pipetted into a scintillation vial and 10 mL of the scintillant Ecoscint A (National Diagnostics) was added. The solution was shaken and left to settle before counting. The activity of ^{65}Zn, ^{109}Cd and ^{64}Cu in 2 mL samples of the filtered supernatant was determined using a gamma counter (1480 Wizard, Wallac).

For determination of E_r, 10 mL of the original, filtered supernatant was transferred into a 15 mL centrifuge tube containing 100 µg of Chelex-100 resin (Bio-Rad Analytical Grade, 100-200 mesh), which had been converted into the calcium form. After 12 h of shaking, the supernatant was discarded and 10 mL of 0.5 M HNO_3 was added to elute the chelex resin. The tubes were returned to the shaker for 1 h. After settling, 2 mL sub-samples were counted for radioactivity as described above.

Metals in the filtered supernatant and the acid eluant from the resin were analysed using ICP-AES (SpectroFlame Modula, Spectro) and, where necessary, GF-AAS (Perkin-Elmer, AAnalyst 600). A further sub-sample of the filtered supernatant was retained for determination of dissolved organic carbon (DOC).

The E values and the resin E values (E_r values) were calculated using the following equation:

$$E_{(r)} = \frac{C_{sol}}{C*_{sol}} \times R \times \frac{V}{W} \qquad \text{Equation 4.1}$$

where E or E_r represent the labile pool of metal (mg kg^{-1}), C_{sol} is the concentration in µg mL^{-1} of the native metal in solution, C^*_{sol} is the concentration in Bq mL^{-1} of the radioisotope remaining in solution after the 3 d equilibration period, R is the total activity of the radioisotope that was originally added to each sample (Bq mL^{-1}), and V/W is the ratio of solution to sample (Hamon *et al.*, 2002 a, Hamon *et al.*, 2002 b, Lombi *et al.*, 2003). For these experiments, the V/W ratio was 10 mL g^{-1}.

4.2.5 Statistical Analysis

Statistical analysis was carried out using the statistical program SPSS. Analysis of variance was used to compare time points and t-tests were used to compare 2 points to each other.

4.3 Results and Discussion

4.3.1 pH

Both oxidation methods resulted in a decreasing pH with increasing E_h (Fig. 4.1, Table 4.1). Slow oxidation in air resulted in a more oxidised sediment (higher E_h) than rapid, peroxide-induced oxidation; yet the rapid oxidation process resulted in a more acidic sediment (Fig. 4.1). Rapid oxidation consistently resulted in lower pH values than the slowly oxidised sediment, for equivalent E_h values. A likely explanation for this is that the slow oxidation method allowed more time for slow sediment processes such as mineral dissolution driven pH-buffering to occur. These slow processes may also account for the greater variability observed for the slow oxidation in air data, compared to the rapid, peroxide oxidation data (Fig. 4.1). Also, addition of the larger volumes of peroxide may have had a direct effect on the pH of the suspension, due its own low pH.

4.3.2 General Lability

Metal lability, as expressed by the E_r value is shown for each metal in Figure 4.2. Lability of all heavy metals were increased in oxidised sediment samples compared to the initial metal lability. An increase in the E_r value is indicative of an increase in the C_{sol}/C^*_{sol} term of equation 1. Hence the native metal in solution is present in a higher proportion with respect to the spiked radioactive metal. An increase in E_r values over the oxidation series (with respect to the E_r value for the untreated sediment) means that a pool of metal which was not labile in the reduced sediment, has become labile due to the oxidation process.

4.3.3 Zinc Lability

The lability of Zn was initially low for both oxidation methods (Fig. 4.2). Slow oxidation over the 48-day period led to Zn lability increasing linearly with decreasing pH, up to a final maximum value of ~1600 mg kg^{-1}, which is ~51 % of total Zn, at pH 4. Peroxide-induced oxidation not only led to a much more rapid decrease of pH with E_h, but also a much more rapid rise in the E_r value. The E_r value increased rapidly to a maximum concentration of approximately 1800 mg kg^{-1} (57 % of total Zn) and remained at this level despite the further decrease in pH. It is likely therefore that this is the maximum potential Zn lability for this sediment. This work shows therefore, that the increase in Zn solubility is not merely a desorption of surface adsorbed Zn in response to oxidation-induced acidification, but dissolution of an initially non-labile solid phase in the sediment, which may be Zn sulphide. The sulphide results presented in Appendix 1 are samples of the WNCC sediment which had been oxidising for 2 months. It is therefore likely that the sulphide content of the samples used in the current experiment, was higher than those values. Acid volatile sulphide was quantified in the WNCC sediment but the data were unfortunately misplaced.

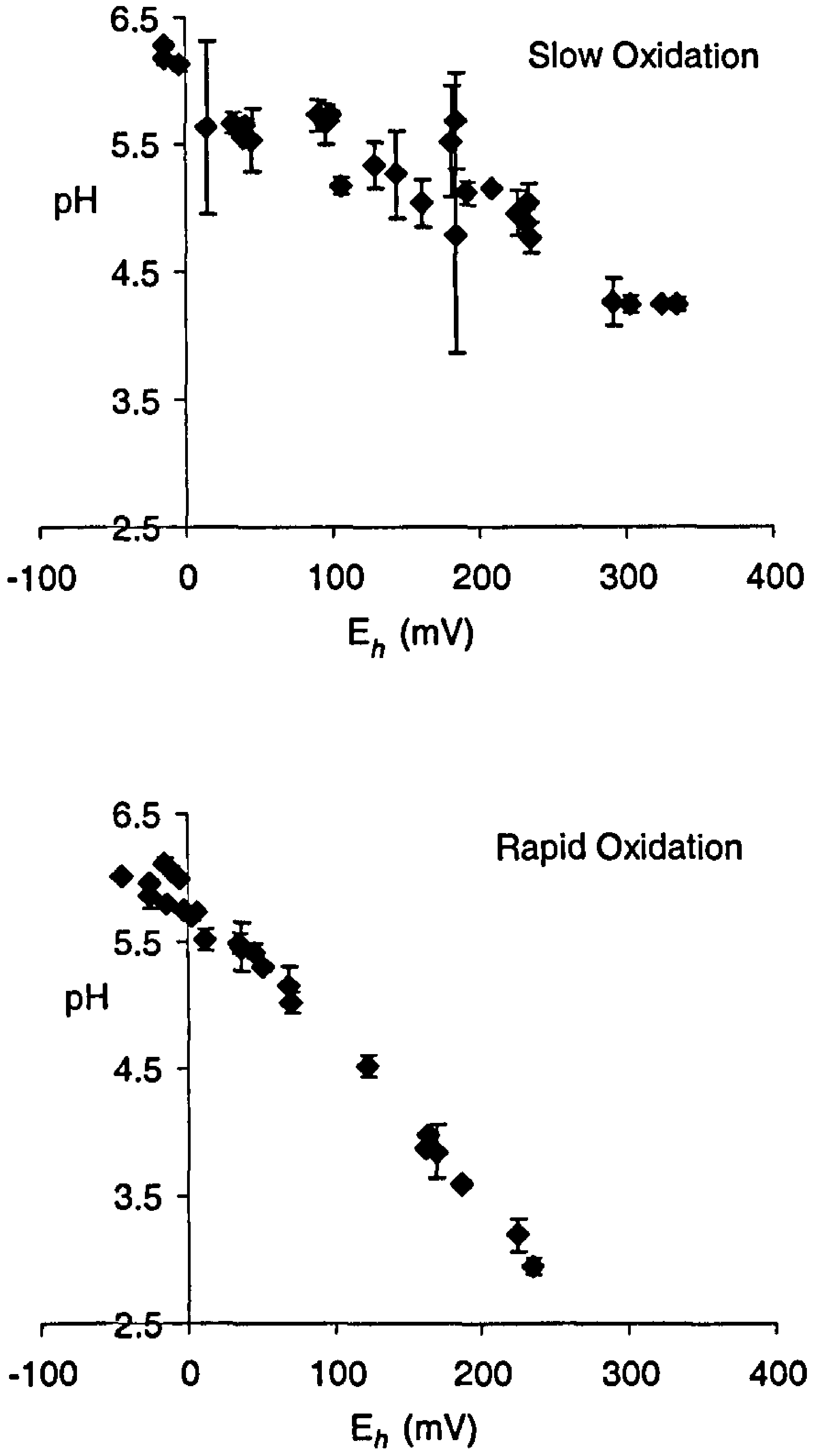
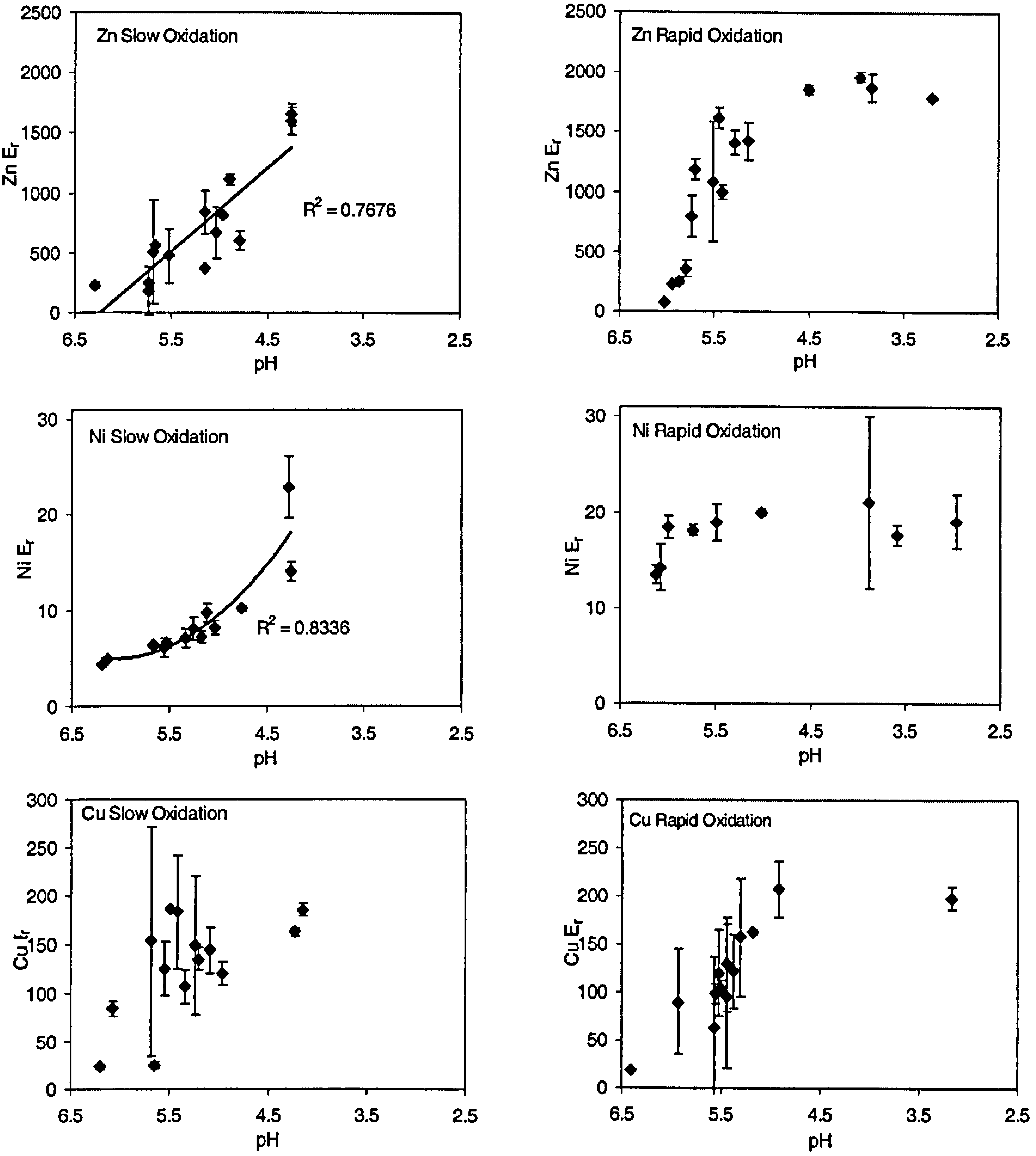


Figure 4.1. The change in pH during "slow" oxidation in air and "rapid" oxidation by hydrogen peroxide addition. Values are mean ± SD (n = 2).



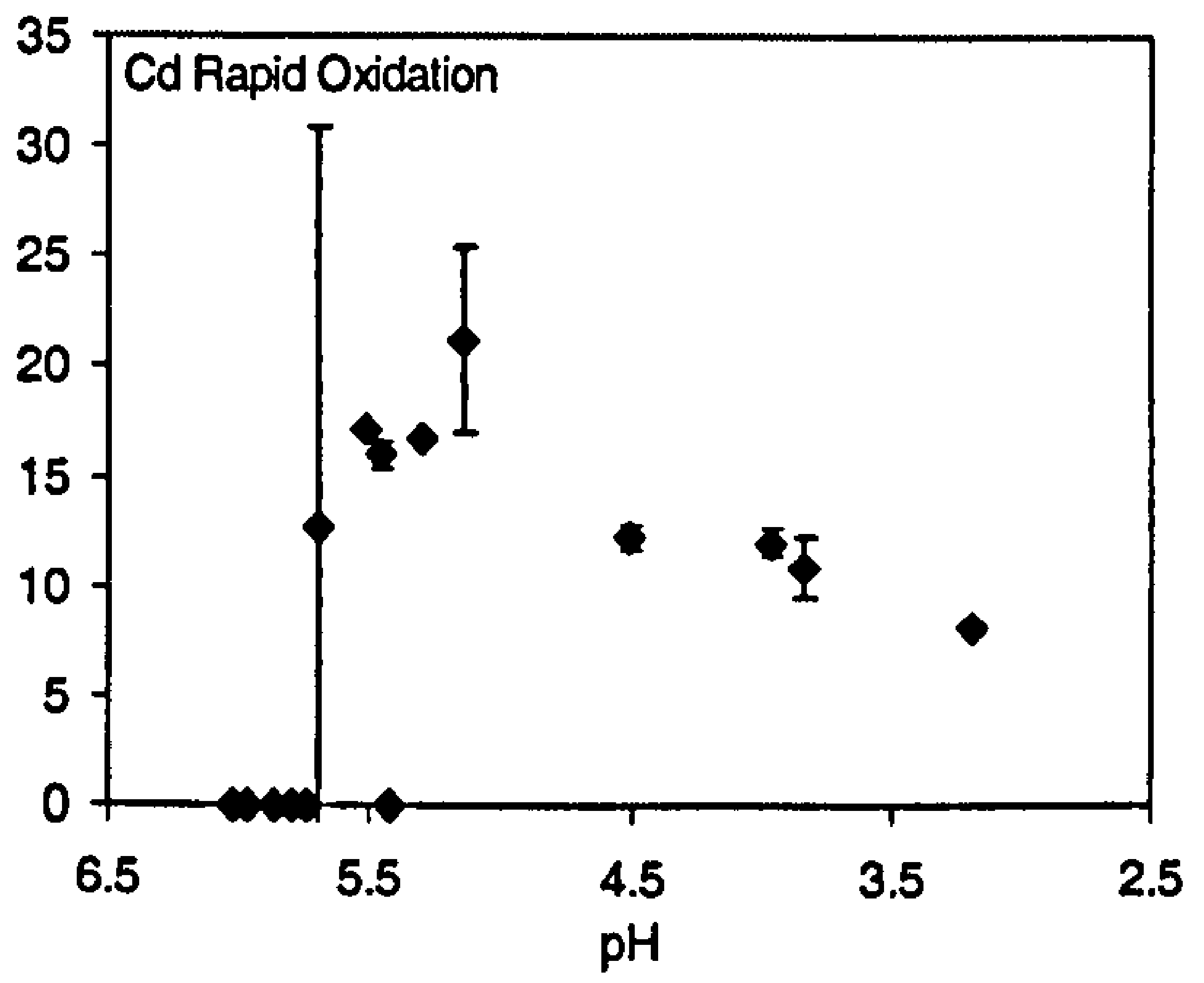


Figure 4.2. The relationship between suspension pH and metal lability (E_r mg kg^{-1}) for rapid and slow oxidation techniques. Values are mean ± SD (n = 2).

4.3.4 Nickel Lability

The lability of Ni showed a different trend with oxidation (Fig. 4.2). During slow oxidation, Ni lability increased with decreasing pH, with large increases in Ni lability observed below pH 4.5. The maximum Ni lability observed was approximately 20 mg kg^{-1} (~25 % of total Ni). Oxidation with peroxide led to the maximum Ni lability being reached at a peroxide addition rate of 17.5 µL g^{-1}. Nickel lability remained at this concentration despite further oxidation. This suggests that the forms of Ni which have the possibility of becoming labile during oxidation are both easily available to, and quick to react with, hydrogen peroxide and are thus liberated at relatively small addition rates. It is possible that they are organic in nature.

These maximum lability values for Ni and Zn are not surprising. Tack *et al.* (1996) found similar results during a study of changing solubility of metals in sediments with pH and differential oxidation. They found maximum solubilities (at pH 1) of ~80 % of total Zn and ~50 % of total Ni (~30 % at pH 3).

Several studies have found Zn and Ni to partition into the operationally-defined carbonate bound/adsorbed phase during sequential extraction procedures for oxidising sediments (Guo *et al.*, 1997, Stephens *et al.*, 2001 a, van Ryssen *et al.*, 1999, Tack *et al.*, 1996). Carbonate can buffer the pH change during oxidation. The carbonate content of the WNCC sediment is negligible, eliminating a major binding phase for the metals. This may cause more metals to bind to the organic molecules present in the system. The addition of hydrogen peroxide will cause these organic phases to be broken down and/or oxidised more rapidly than in the slow oxidation experiment and, coupled with the lesser mineral phase buffering, may explain the observed differences in labilities between the two methods.

4.3.5 Copper Lability

Copper lability increased throughout both methods of oxidation (Fig. 4.2). However, reproducibility, though good at the extremes (little or no oxidation, and maximum oxidation), was poor in the intermediate oxidation conditions. The maximum labilities of Cu were similar after slow (30 % of total) and rapid (33 % of total) oxidation. As with Zn and Ni, lability seemed to increase more rapidly with respect to pH during the rapid oxidation experiment. Maximum lability was reached at pH 4.9 in the rapid oxidation experiment, but not until pH 4.2 during slow oxidation. Addition of 50 μL g^{-1} of peroxide was enough to yield maximum lability of Cu. Addition of 1000 μL g^{-1} peroxide reduced the pH to below 3.5, but did not increase the Cu lability any further. This is very similar to the behaviour of Zn during rapid oxidation.

4.3.6 Cadmium Lability

It was not possible to calculate E values for the full oxidation range for Cd as, in many cases, the solution activity of the ^{109}Cd isotope was too low to be counted above the background Zn activity. During the slow oxidation process, E_r values were obtained for 41 and 48 d of oxidation only (data not shown). At 41 d Cd E_r was 11.9 (56 % of total) and at 48 days E_r was 12.3 (57 % of total). Rapid oxidation with peroxide yielded E_r values below about pH 5.5 (Fig. 4.2). Maximum Cd lability (~84 %) occurred at around pH 5.5 and dropped steadily with increasing oxidation. This was an unexpected trend, but is in agreement with the results presented in Chapter 3. It may be that Cd is co-precipitating with iron oxides as they form.

4.3.7 Colloidal Occlusion

The four metals showed different behaviour with respect to occlusion by colloids (Fig. 4.3). In general, Zn showed little or no occlusion within colloids and the E/E_r ratio remained close to 1. The untreated sediment used in the rapid oxidation experiment yielded a Zn E/E_r value of 2. It is likely that this is due to occlusion of Zn in inorganic colloids, which may be Zn sulphides. Nickel however, showed some occlusion within colloids for both methods of oxidation. When oxidised slowly in air the E/E_r ratio dropped slowly down to 1 from an initial value of approximately 1.4. Hence initially, approximately 30 % of the labile metals were occluded in colloids. It was observed that natural oxidation in air of the supernatant that had been filtered to < 0.2 μm, resulted in the flocculation of colloids after a few days of storage. During oxidation of the sediment, these oxidation and

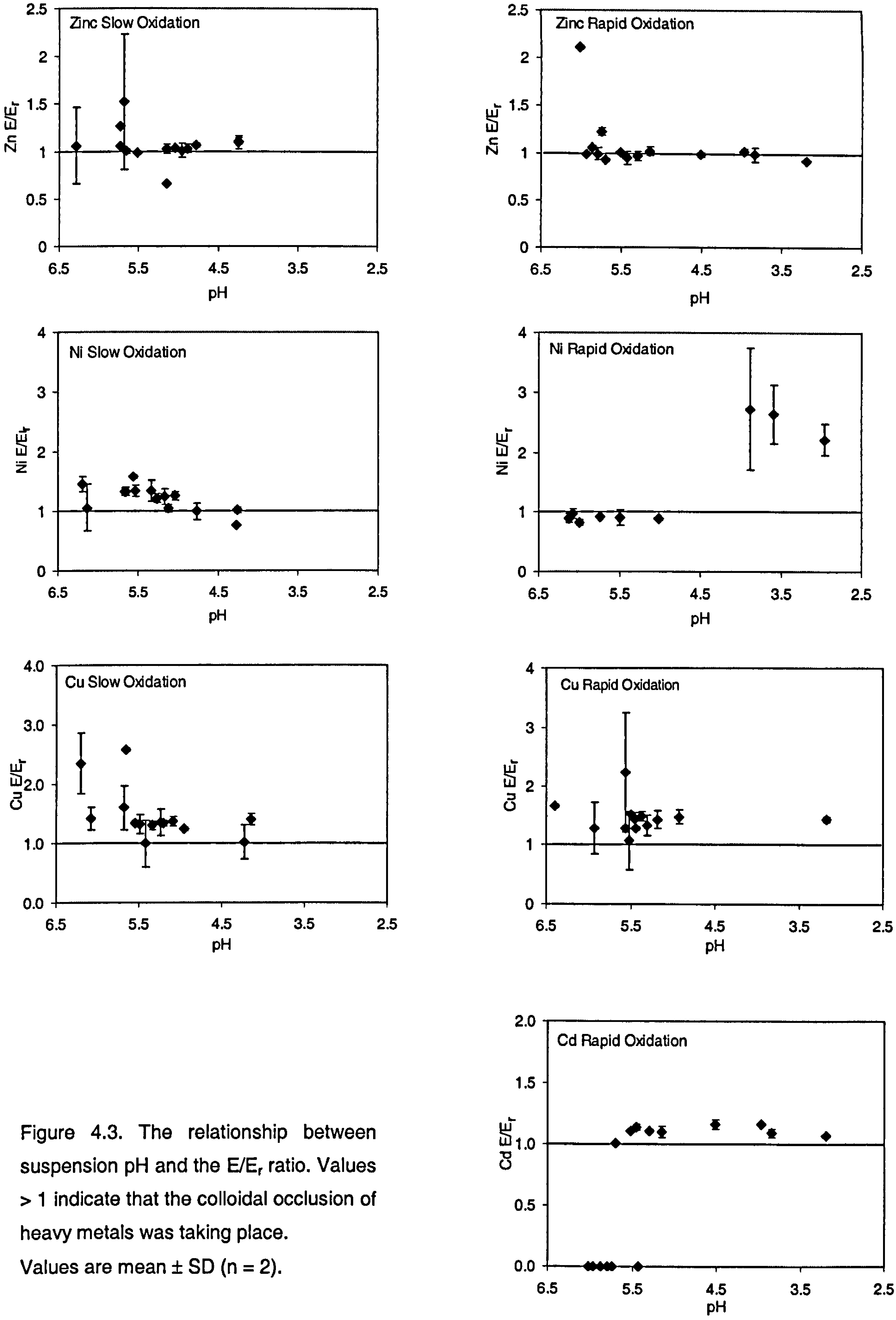


Figure 4.3. The relationship between suspension pH and the E/E_r ratio. Values > 1 indicate that the colloidal occlusion of heavy metals was taking place.
Values are mean ± SD (n = 2).

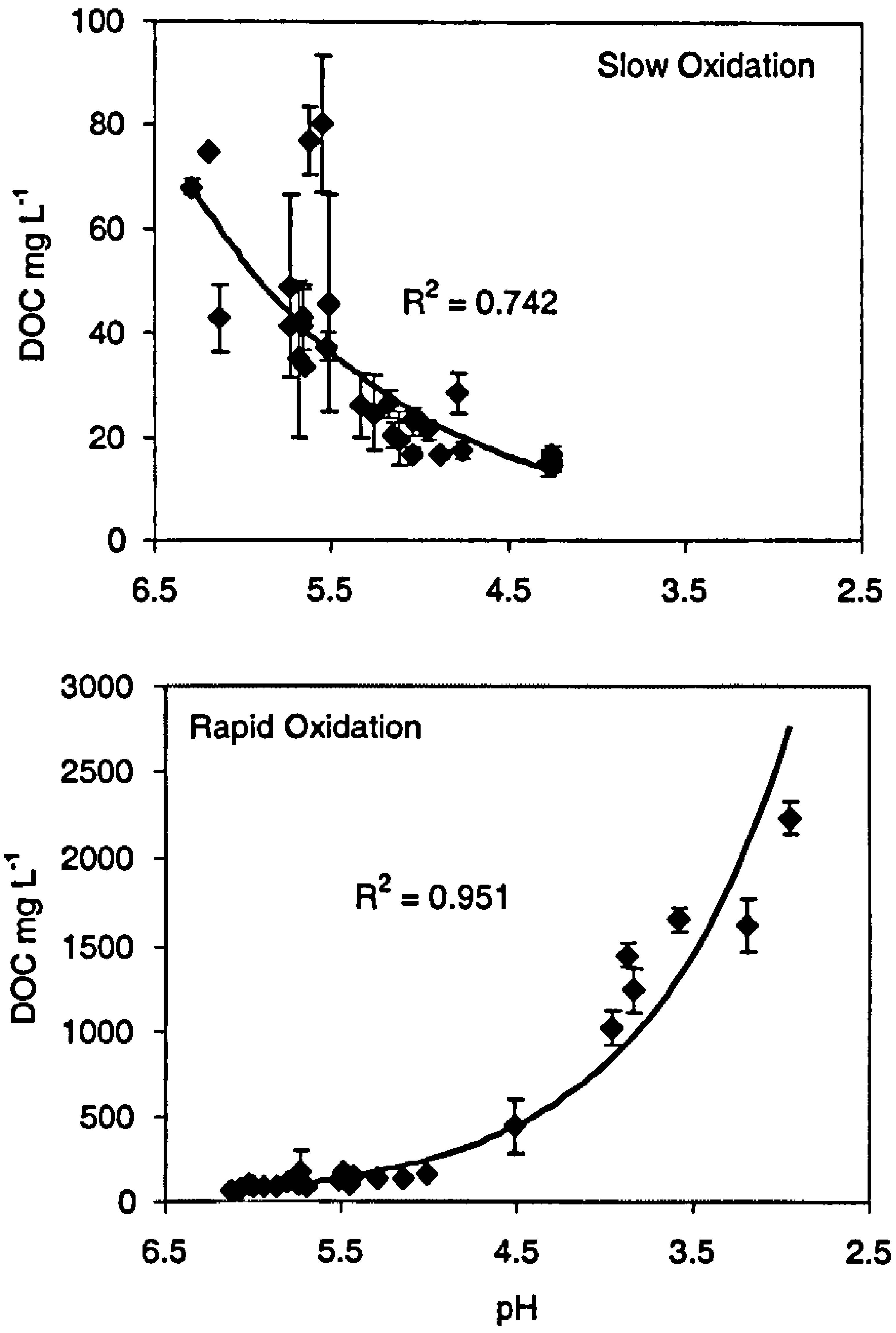


Figure 4.4. The relationship between pH and DOC during "slow" oxidation in air and "rapid" oxidation using peroxide. Values are mean ± SD (n = 2).

precipitation processes will occur in the pore water, hence the associated decrease in DOC (dissolved organic carbon) seen in Figure 4.4. Perhaps the decrease of the E/E_r ratio down to 1 occurs as the colloids occluding the Ni flocculate and settle out of solution and therefore are not included in the E value measurements. This effect is more pronounced in the Cu slow oxidation data (Fig. 4.3), which showed a pattern of changing E/E_r with respect to pH, very similar to that for DOC during slow oxidation (Fig. 4.4). It is therefore very likely that it is these dissolved organic molecules that are occluding the Cu.

Rapid oxidation with hydrogen peroxide showed colloidal occlusion of Ni only at the lowest pH values, and hence the highest addition rates of peroxide. Peroxide addition to the suspension will oxidise the organic and inorganic colloids present in anaerobic conditions, causing them to precipitate. Subsequent increases in DOC with peroxide addition are likely to be due to the breakdown of larger organic molecules present in the sediment. This breakdown could have led to the liberation of smaller organic molecules, capable of binding metals in non-exchangeable forms, hence the increase in the E/E_r ratio at low pH. It is likely that addition of even small volumes of peroxide caused a release of metal occluded by the colloids. As the addition of peroxide was into a suspension, it is likely that the colloids were amongst the first sediment constituents to react with the peroxide. Thus, no occlusion was observed until the peroxide had liberated a large amount of organic carbon into solution (Fig. 4.4. Note the different scales on the Y-axes). DOC during slow oxidation exhibited the same pattern as the Ni E/E_r ratio with pH change. It is therefore probable that organic colloids were responsible for occluding Ni, though at the higher pH values inorganic colloids may also have been present. It is almost certain that it was organic colloids that were occluding Ni in the rapid oxidation experiment, since at such low pH values, inorganic colloids are unlikely to be present.

Due to the difficulties in obtaining E and E_r values for Cd, it was not always possible to derive an E/E_r ratio. The E/E_r values, when available, were very consistent throughout oxidation, generally being between 1.1 and 1.2. This shows that there is some significant occlusion of Cd by colloids.

4.4 Conclusions

Labilities of the metals after complete oxidation by either hydrogen peroxide addition or oxidation in air were similar, but metal behaviour before this end point was different. Rapid oxidation using peroxide tended to slightly overestimate the amount of metal which was released upon sediment oxidation in air, and could therefore be used as a conservative test to assess potential metal release. It is interesting to note that almost two thirds of the total Ni and Cu in the sediment remained non-labile, even when fully oxidised and acidified to pH values < 3.5, whilst almost 57 % of the total sediment Zn and 84 % Cd was mobilised. This illustrates that total sediment metal concentrations are not a good basis on which to assess potential release across all metals. Overall, oxidation significantly increased labilities of Zn, Ni, Cd and Cu. These results prove that the fractionation changes that were shown in Chapter 3 to occur during oxidation, are leading to an increase in lability and hence bioavailability of the heavy metals in the WNCC sediment.

CHAPTER 5

Root Elongation of *Brassica napus* L. and *Lolium multiflorum* L. Over an Oxidation Series on a Contaminated Sediment

5.1 Introduction

Phytotoxicity of heavy metals in soils and sediments depends on their bioavailability. Bioavailability is affected by a range of soil/sediment physical (e.g. structure, particle size distribution), chemical (e.g. pH, E_h, total concentration, fertility) and biological (e.g. mycorrhizae, bacteria) factors (Ernst, 1996). The higher the bioavailability of a heavy metal, the greater the phytotoxicity will be. In the field, large-scale experiments can be conducted using a wide range of species, such as trees. Phytotoxicity of tree species in the field will be discussed later in this thesis (Chapter 7). In the present Chapter, the phytotoxicity of the sediment was examined in the laboratory through the use of a root elongation study. The root elongation study was carried out on sediment oxidised over a period of up to 48 days to quantify the phytotoxicity associated with the heavy metal mobilisation described in Chapters 3 and 4. Two experimental species were used: concorde ryegrass (*Lolium multiflorum*) and canola (*Brassica napus*).

5.1.1 Root Elongation and Heavy Metals

Phytotoxicity of pollutants can be measured in a variety of different ways. Laboratory based phytotoxicity assays may focus on germination, root elongation, early seedling growth and/or life-cycle bioassays (Ernst, 2003). Root elongation assays avoid the difficulties associated with germination assays, namely: differing population and species-specific responses, impermeability of seed coats, and precipitation or complexation of chemicals in the cell walls (Ernst, 2003). Wong *et al.*, (1981) report that root elongation was a more sensitive test than seed germination. Cell division and root elongation are often adversely affected when exposed to increased concentrations of chemicals (Ernst, 2003). Many species, both terrestrial and aquatic, have been utilised in phytotoxicity testing. Reviews of the use of aquatic plants for phytotoxicity testing are provided by Lewis, (1995) and Mohan and Hosetti, (1999). However, the phytotoxicity of contaminants to terrestrial plants is of the most interest in the current study as the WNCC sediment was dredged to form a planting platform at the field site. Wetland plants were not used in this study as the aim of remediation of the WNCC site would be to dredge and dry the sediment and allow it to colonise with grasses and local dicotyledenous species. *Brassica napus* is well represented in the literature and *Lolium multiflorum* was chosen for consistency, as it had been used previously in the experiment described in Chapter 6.

Contaminated sediments may not necessarily have a phytotoxic effect when used as a land application. Chen *et al.*, (2002) report that a sediment acted to improve biomass production in *Brassica chinensis* (Pakchoi) when applied at a rate of < 540 t ha^{-1}. Application of sediment at above this rate however, led to a decrease in biomass production compared to the control, due to toxic effects of heavy metals. The organic pollutants present in the WNCC sediment are also likely to exert an effect on root elongation (Wong *et al.*, 1981). Lead can cause significant decreases in root elongation and led to shorter, thicker roots in lupins (Rucińska *et al.*, 1997). The effect of Cu on root biomass of *Brassica napus* has been reported to be greater than the effect of Zn, though in combination, toxicity was synergistically increased (Ebbs and Kochian, 1996). Copper and Zn inhibited lateral root development in *B. napus*, and Zn led to a decrease in lateral root diameter. Aqueous sludge extracts have been shown to retard both germination and root elongation in *Brassica parachinensis* (Chinese cabbage) and *Raphinus sativus* (radish) (Wong *et al.*, 1981). Thus it should be borne in mind that leachate from the WNCC planting platform may adversely affect surrounding flora (Bedell *et al.*, 2003). Thus, heavy metals can have toxic or inhibitory effects on plant growth and metabolism, cell division and root elongation. However, Al^{3+} can also have toxic effects on plants. Roots are the plant organs most sensitive to Al toxicity and experience rapid inhibition of main axis elongation and lateral root development in the presence of Al (Barceló and Poschenrieder, 2002). This decrease in root development and elongation can be due to a variety of reasons, namely Al-induced inhibition of cell division, root cell elongation, Ca metabolism and phosphate availability (Barceló and Poschenrieder, 2002). Young seedlings are more susceptible to Al toxicity than older plants (Foy *et al.*, 1978). For this reason, Al has been included within the scope of the present study.

An advantage of using *Brassica napus* in the current experiment is that, unlike some other families, *Brassica* roots are not infested with mycorrhizal fungi and thus a potentially complicating factor is removed.

5.1.2 Aim

- To determine if changes in metal fractionation and lability over a 48-day oxidation period (as described in Chapters 3 and 4) constitute an increase in metal bioavailability and phytotoxicity.

5.1.3 Hypothesis

The anoxic sediment will prove fatal to the seedlings as the roots will be unable to respire. Sediment that has been partially oxidised to the extent that it is oxic, but still contains a significant moisture content, will allow root elongation. The sediment that has been more thoroughly oxidised will have high concentrations of heavy metals, liberated by the oxidation process and the accompanying low pH. These conditions will prove toxic to the plant species and root elongation will decrease with respect to that seen in partially oxidised sediment. It is expected that, after oxygen penetration into the sediment has taken place, root elongation in the plant species will decrease more with increasing oxidation time until the point of maximum metal lability and minimum sediment pH is reached. At this point, death of seedlings may occur.

5.2 Material and Methods

5.2.1 Sediment

Sediment used in the current study was freshly removed (after homogenisation) from the storage barrel from which the sediment used in Chapter 3 and Chapter 4 had also been taken. The sediment had been covered with a layer of native canal water to minimise oxidation during storage. The sediment sampled for use in the current experiment was stored in a polyethylene tub at 4 °C, covered by a layer of native water, until needed.

5.2.2 Experimental Set-up

Preparation for the experiment involved allowing sediment samples to oxidise for differing periods between 0 and 48 days (Table 5.1). Nine time intervals were used, which coincided with those used in previous work (Chapters 3 and 4). At each time point, the sediment in the tub was thoroughly homogenised by hand and a 1 cm deep layer of sediment (approximately 200 – 210 g wet weight) was added to a cling film-lined aluminium tray. Lids were loosely fitted to the trays to minimise evaporation, but allow full gas exchange. Time intervals were synchronised so that oxidation of all sediment samples finished on the same day i.e. the first sample to be laid out was the "48 day" sample, and this was laid out 48 days before the oxidation period finished. The "41 day" sample was laid out 7 days later, and so on. (The "0 day" sample was not laid out at all before the end of the oxidation period, but was weighed directly into petri-dishes). On the final day of oxidation, subsamples were taken from each tub for determination of moisture content. The sediment was then frozen until needed, to halt oxidation.

Prior to the root elongation study, sediment samples were defrosted at room temperature overnight then homogenised and sieved to < 2 mm. Sediment equivalent to 10 g dry weight was transferred into petri-dishes. The remaining sediment was re-frozen for future determination of pH and heavy metal mobility. Duplicate petri dishes were set up with sediment from each time point and each control treatment. Where the moisture content of the WNCC sediment was below field capacity

(93 % water), deionised water was added until this value was reached (Table 5.1). Four different control treatments were used: an uncontaminated pond sediment dredged from Ness Botanical Gardens at field capacity (FC) and at saturation (170 % water), and a compost (John Innes no. 2) at FC and saturation (120 % water). Saturated control treatments were used to allow quantification of the effect of sediment saturation on root elongation. Thus, adjustment to the WNCC sediment results could be made if the effect of the waterlogging itself proved to have a significant effect on root elongation. Petri dishes were then left in the dark at 20 °C for 24 hours prior to seedling addition.

5.2.3 Experimental Plants

The root elongation of *Brassica napus* and *Lolium multiflorum* were examined using a modification of the method of Crawford and Wilkens (1998). Seeds were left to germinate in the dark at 20 °C, on damp tissue paper for 36 hours, in excess of the number required for the experiment. Seeds with root radical lengths of between 0.5 and 2.0 mm were selected (henceforth referred to as "seedlings"). All seedling root lengths were measured by hand, to the nearest 0.5 mm before addition to triple-vented petri dishes. Three seedlings of each species were laid on the sediment surface in each petri dish and gently pushed to ensure contact with the sediment. Thus each dish contained 6 seeds, and the seeds of both species were subject to the same conditions. The seeds were numbered on the lid of the dish and the lid was fixed in place with a small piece of sellotape. The lids were attached so as to ensure that vents were open to allow efficient gas exchange. The petri dishes were then returned to the controlled temperature environment and left undisturbed for 72 hours in the dark at 20 °C. A beaker of water was left with the petri dishes to maintain air humidity and help prevent desiccation or excessive drying of the sediment and seedlings. Seedling addition to all petri-dishes took place on the same day and thus all samples experienced the exact same conditions throughout the 72-hour growth period. After 72 hours, all root lengths were re-measured to the nearest 0.5 mm. For each time point and control treatment, root elongation data had therefore been collected for 6 seedlings of each species. These data were pooled by species, and averaged. Observations of the number of roots which had grown, and their degree of penetration of the sediment, were recorded before seedlings were removed from the petri dishes.

Table 5.1 Moisture content (g $100g^{-1}$, or percentage) and pH of the sediment and controls. Sediment with a moisture content below field capacity (93 g $100g^{-1}$) were adjusted to this level. Control treatments were made up to field capacity (FC) and saturation from air-dried soil/compost.

Days of Oxidation	**Moisture Content (g $100g^{-1}$ DW)**	**Adjusted to (g $100g^{-1}$ DW)**	**pH**
0	183	-	6.16
6	177	-	5.69
17	170	-	5.59
21	164	-	5.52
28	124	-	5.43
31	55	93	4.53
35	56	93	4.57
42	50	93	4.46
48	39	93	4.17
Control			
Ness FC	air dry	60	6.98
Ness 170 %	air dry	170	
Compost FC	air dry	67	6.21
Compost 120 %	air dry	120	

5.2.4 Sediment Physical and Chemical Analysis

Sediment moisture content was determined by the drying of subsamples at 105 °C for 24 hours. The following procedures were carried out on the sediment which had previously been re-frozen. Sediment was allowed to defrost overnight on the bench-top before use. Sediment pH was measured in a 1:5 sediment-to-water suspension, which had been shaken for one hour and allowed to settle for half an hour. A two-step water-NH_4Cl sequential extraction was carried out as described in Chapter 3. Metal concentrations in extracts were determined using ICP-AES as described previously.

5.2.5 Statistical Analysis

Statistical analysis was carried out using the statistical package SPSS. Kruskall-Wallis analysis was used for comparing > 2 variables. For comparison of 2 variables, Mann-Whitney U tests were employed. The p-values quoted in this chapter are derived from these tests.

5.3 Results and Discussion

5.3.1 Root Elongation

Root elongation of *Brassica napus* and *Lolium multiflorum* planted on the control treatments are shown in Figure 5.1. Neither species exhibited significantly different root elongation between control treatments (Oneway ANOVA $p > 0.05$). Therefore, no alteration has been made to the root elongation data obtained for the WNCC sediment samples to account for the effect of differing moisture content. Root elongation of the experimental plants growing in the WNCC sediment is shown in Figure 5.2. These data are expressed as percentage of the mean root elongation of the controls for each species, which were 35.22 ± 14.10 mm and 41.01 ± 21.27 mm (mean ± SD) for *L. multiflorum* and *B. napus* respectively. The roots of *B. napus* died in sediment that had been oxidised for 21 days or less. This is indicated in Figure 5.2 by a negative root elongation, as the existing length was lost. Positive root elongation of *B. napus* roots occurred in sediment that had

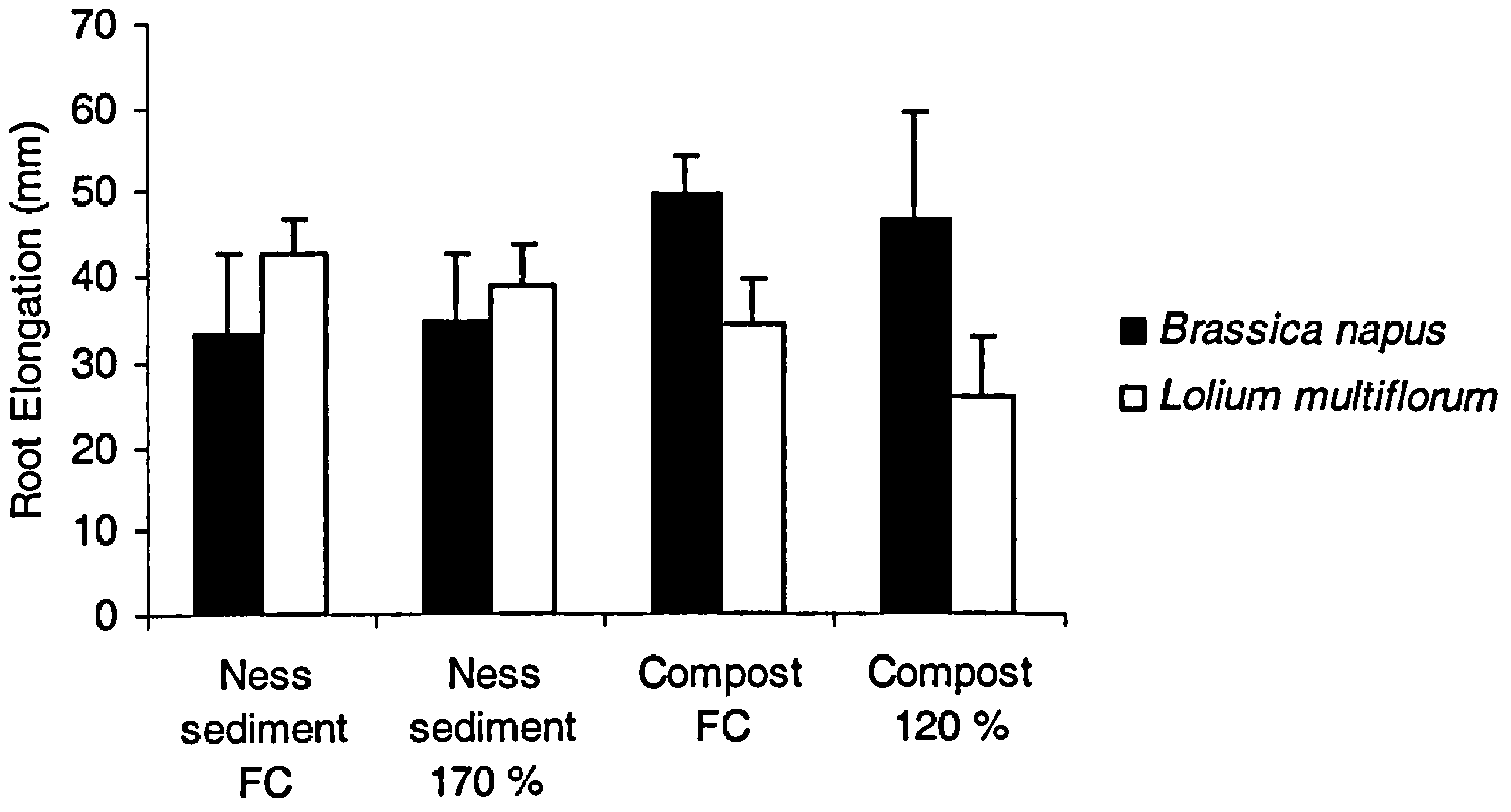


Figure 5.1 Root elongation in *Brassica napus* and *Lolium multiflorum* in the four control treatments. FC = field capacity. Values are mean + SE (n = 6). One-way ANOVA shows no significant intra-species difference between control treatments ($p > 0.05$).

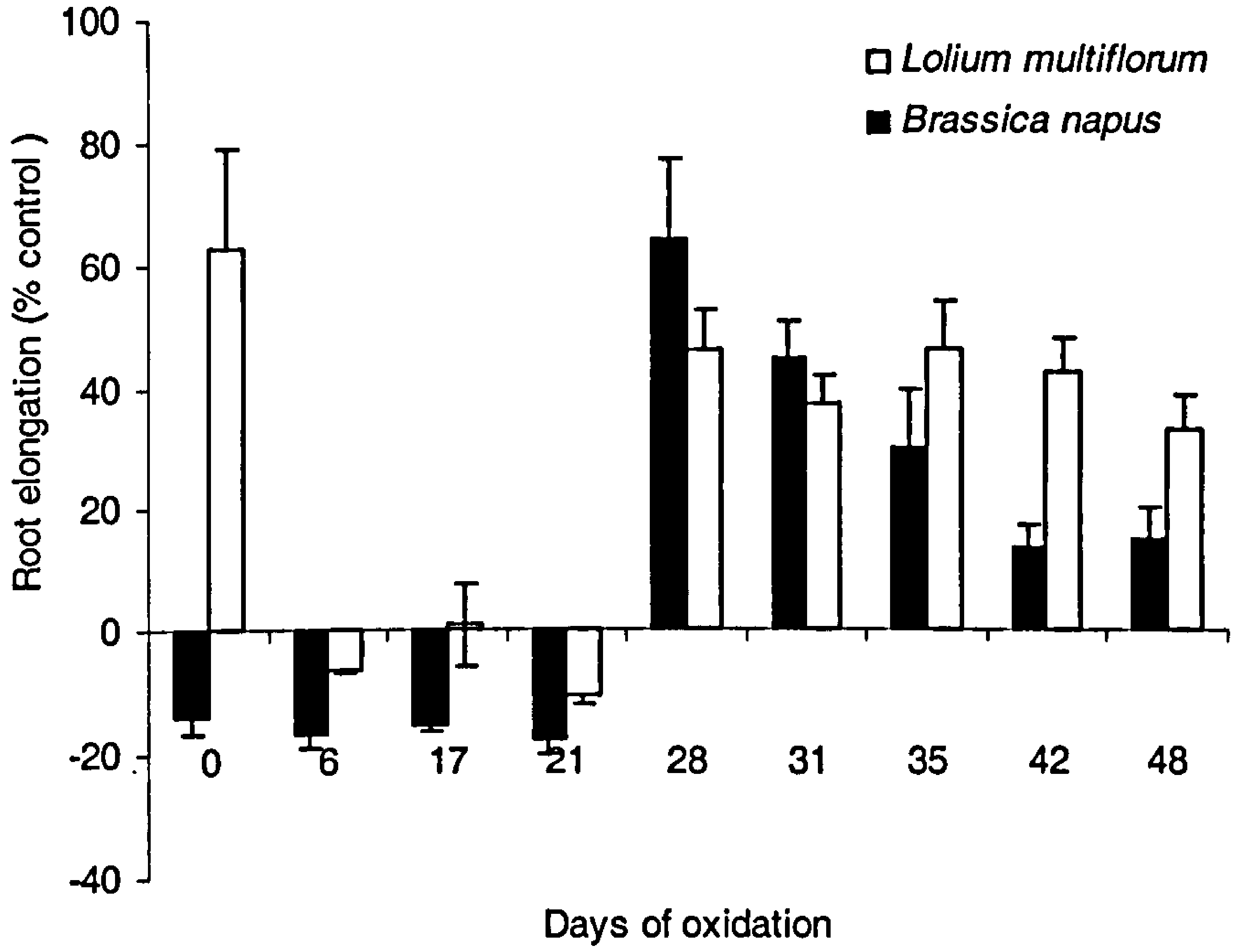


Figure 5.2 Root elongation in *Brassica napus* and *Lolium multiflorum* over 72 hours, in sediment which had been oxidising for varying times between 0 and 48 days. Values are shown as a percentage of the mean growth of control treatments (Fig. 5.1) for each species. Mean elongation of *Lolium* roots was 35.2 mm and *Brassica* roots was 41.0 mm. Note that sediment oxidation continued during the 72 hours of the root elongation trial. Values are mean + SE (n = 6).

been oxidised for 28 days or longer. Maximum root elongation (~65 % of the control value) occurred at this 28-day point. After that time root elongation decreased with respect to the 28-day value. The decrease in root elongation was significant ($p < 0.001$) between the sediment that had oxidised for 28 days and that which had oxidised for 48 days. The maximum root elongation in *L. multiflorum* (~ 65 % of the control value) occurred in sediment that had not been oxidised before the 72-hour growth period had begun. In sediment samples that had been oxidised for between 6 and 21 days, negative or barely-positive root elongation occurred. Root elongation in *L. multiflorum* was next positive in the sediment that had been allowed to oxidise for 28 days prior to the start of the growth period. From 28 to 48-days of oxidation there was no significant difference in root elongation of the *L. multiflorum* seedlings.

In sediment that had oxidised for between 6 and 21 days, a negative root elongation occurred due to death of the existing root during the course of the experiment. This was thought to be due to the lack of oxygen reaching the roots due to the water-saturated conditions. The two species showed different rooting behaviour. *B. napus* tended to root directly down into the sediment whereas *L. multiflorum* frequently grew roots across, or looping above, the surface of the sediment. This difference in behaviour explains the differences in root elongation on the anoxic sediment that had experienced no oxidation (0 days). The *B. napus* roots entered the sediment directly and failed to survive in the anoxic, water saturated conditions, whereas the *L. multiflorum* roots tended to grow above the surface, thus avoiding the wet, anoxic sediment. The sediment that had oxidised for between 6 and 21 days exhibited the highest phytotoxicity to both species, and the sediment which had experienced no oxidation (0 days) was also very phytotoxic to *Brassica napus*. During this time the pH of the sediment was relatively high, and the heavy metal mobility low (Fig. 5.4, Chapters 3 and 4). The phytotoxicity observed during this time was most probably due to lack of oxygen to the plant roots owing to the anoxic nature of the sediment.

At 6 days of oxidation, the sediment was dry enough to allow formation of an oxidised surface layer during the course of the 72-hour elongation period. Below this layer the sediment was still black and anoxic. The surface layer is likely to have become more toxic due to the decrease in pH and increased heavy metal lability (see results from Chapters 3 and 4). Proof that the surface layer was oxidising is provided by the observation that it became orange in colour. Penetration below this layer will probably have resulted in root suffocation and death. Anoxia to the root is therefore still thought to be the main cause of root death at this time-point.

Between 21 and 28 days of oxidation the sediment moisture content decreased by 40 % from 164 % to 124 % moisture (Table 5.1). This drying would have allowed greater oxygen penetration to the deeper levels of the sediment, thus allowing the roots to penetrate the surface layer and survive in the deeper layers in the drier sample. The redox potential of the sediment was not measured in the current experiment but the results obtained from the experiment described in Chapter 4 are shown in Figure 5.3. During that experiment, sediment was also oxidised in a layer 1 cm thick as in the current experiment. (Redox potential was measured using a redox probe, in a suspension of 2 g sediment to 20 mL oxygenated MilliQ water with 2 drops of toluene also added). Thus the redox potential is expressed in Figure 5.3 as a relative redox potential, rather than as a true value for the sediment alone. Redox potentials expressed in Figure 5.3 are likely to be more positive than the redox potential in the un-diluted sediment. Throughout the 48-day oxidation period, a large increase in redox potential occurred (Fig. 5.3). In the current experiment, the same pattern of increasing oxidation with time would be expected, and is confirmed by the associated decrease in pH (Table 5.1), which has been discussed previously (Chapter 4, Fig. 4.1).

The greatest elongation in the roots of *B. napus* was seen at 28 days. *B. napus* then showed a significant ($p < 0.001$) decrease in root elongation with time. Elongation in the *L. multiflorum* roots was at no time as great as at 0 days. However, as the roots mostly grew out of the un-oxidised (0 day) sediment and looped into the air, this result must be discounted as the root elongation *into* the sediment is of interest in this chapter. *L. multiflorum* did not show a significant change in root elongation ($p = 0.506$) over the period from 28 to 48 days of oxidation, though roots did penetrate and grow into the sediment at all time-points.

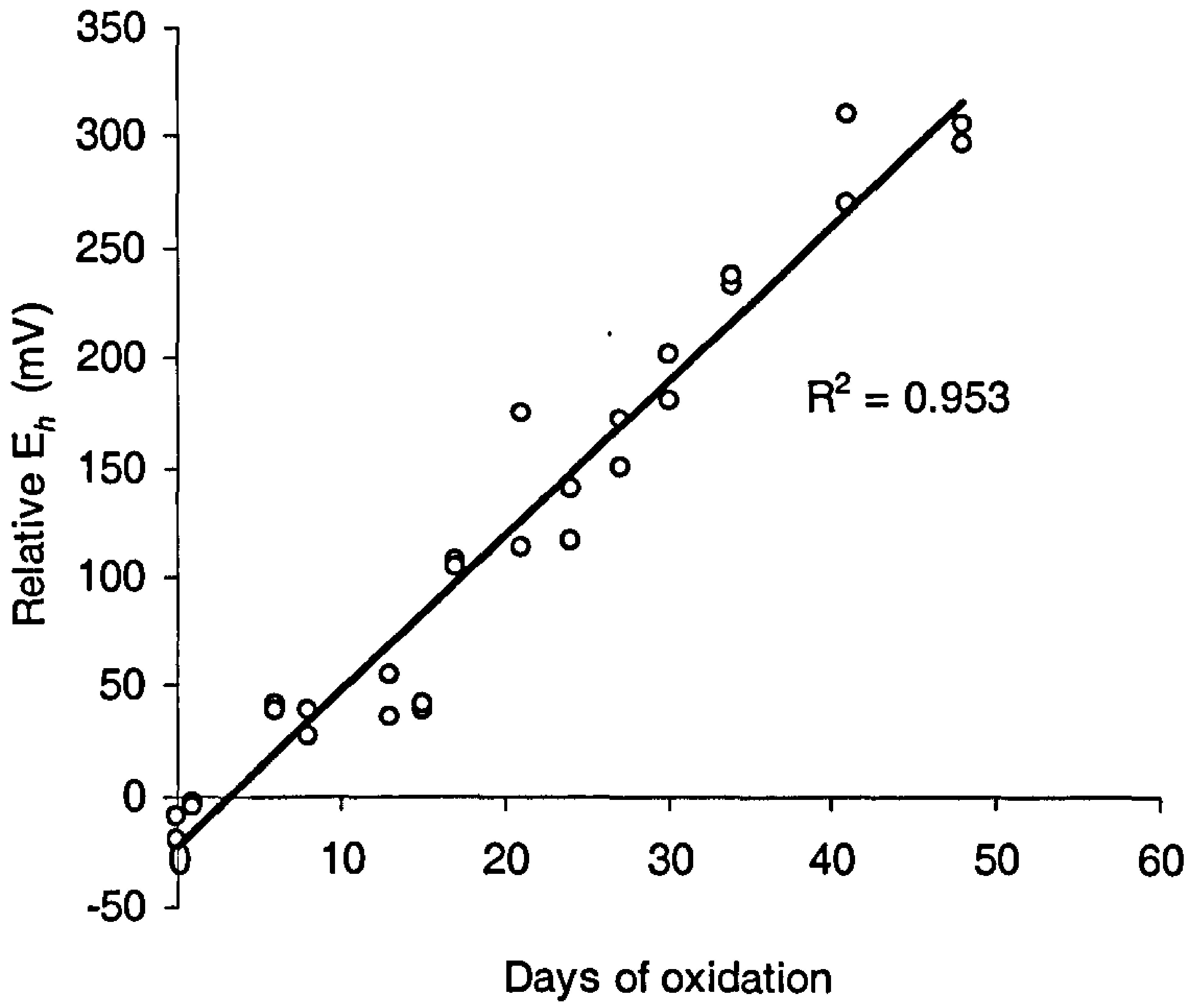


Figure 5.3 Relative redox potential (E_h) of a suspension of 2 g dry weight equivalent of sediment in 20 mL oxygenated, MilliQ water with a N_2 purged headspace after 3 d shaking on an end-over-end shaker. Values are Chapter 4 experimental data from sediment oxidised over a 48 days period in a layer with a thickness of 1 cm.

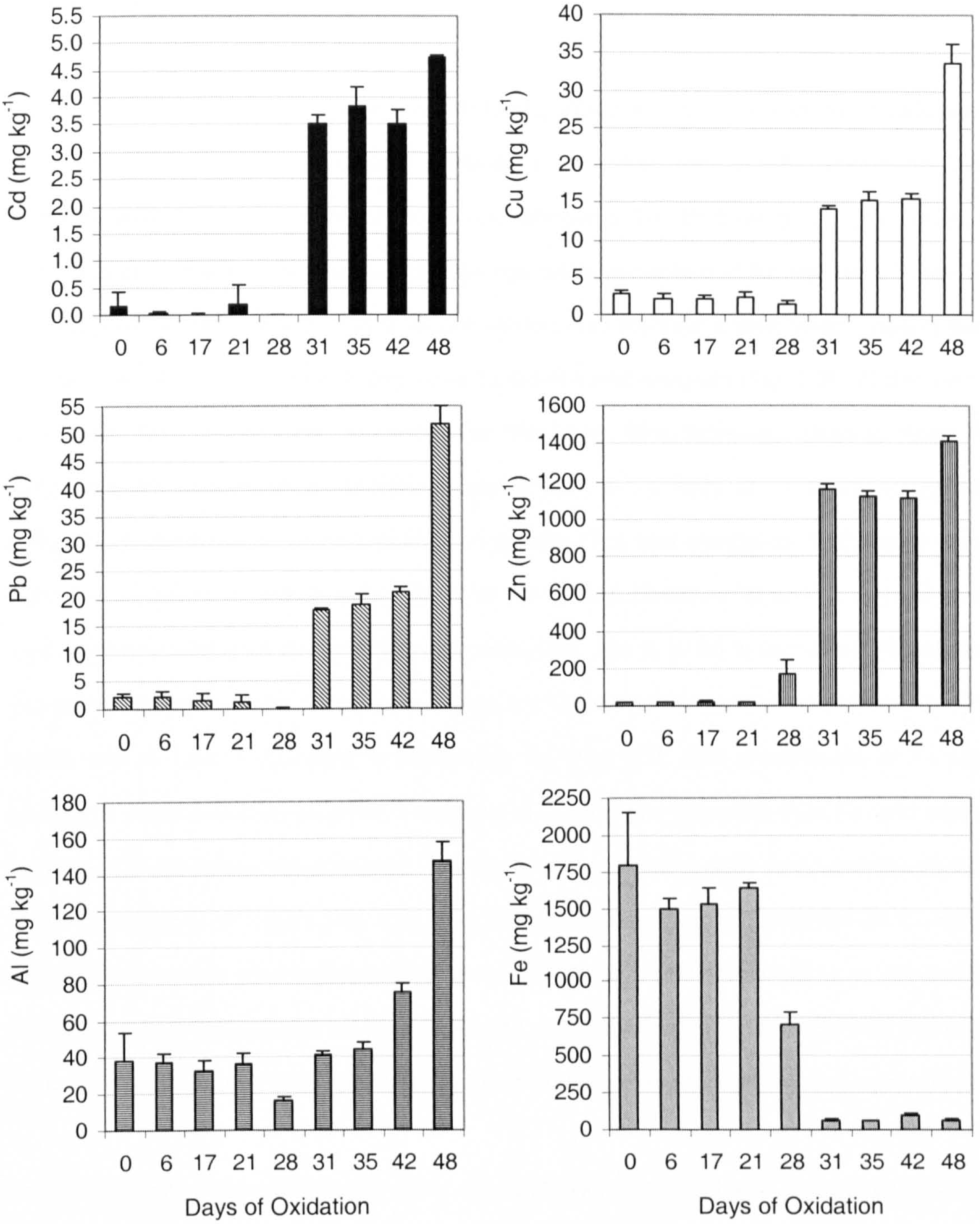


Figure 5.4 Heavy metals extracted from the oxidising sediment upon which the root elongation study was carried out. Extraction was via a two-step sequential extraction. Extracts of the two steps (distilled water and 0.1 M ammonium chloride) were combined and acidified before analysis. Values are mean + SD, (n = 3).

5.3.2 Metal Availability Changes

Heavy metal extractability increased with increasing oxidation (Fig. 5.4). This was as expected and as discussed in previous chapters. Extractable (in water and 0.1 M ammonium chloride) concentrations of Cd, Cu, Pb and Zn were low between 0 and 28 days of oxidation, owing to the more reduced conditions and the high percentage moisture content of the sediment (Table 5.1). At 28 days of oxidation, the sediment moisture content had reached a level which allowed sufficient oxidation for the *Lolium* and *Brassica* roots to survive and elongate (Fig. 5.2). At this point also, extractable Cd, Cu, Pb and Al tended to decrease. Zinc however, became more mobile. Extractable Fe concentrations decreased significantly at 28 days of oxidation, suggesting the precipitation of Fe oxides in the presence of oxygen. The root elongation of *B. napus* was at its maximum at this time, though only ~ 65 % of the control. Between 28 and 31 days of oxidation, very significant drying of the sediment occurred (from 124 % to 55 % moisture). Associated with this drying and increased oxidation was a large and significant increase in extractability of all heavy metals and Al, and a decrease in extractable Fe (Fig. 5.4). The precipitation of Fe suggests increasing redox potential. Sulphate is reduced at lower redox potentials than Fe, and thus will be oxidised at lower redox potentials also. The higher heavy metal mobility increased the phytotoxicity of the sediment to *B. napus* (Fig 5.2). The phytotoxic effects of the sediment on *L. multiflorum* during this time was less certain owing to the differential rooting behaviours of the species. *L. multiflorum* tended to produce multiple side roots, even when death of the main root had occurred. Thus the elongation was not as clear-cut as in the *Brassica*, which produced only one main root.

The very sudden increase in heavy metal extractability between 28 and 31 days of oxidation and the increase again from 42 to 48 days of oxidation, did not mirror the pattern of extractability presented in Chapter 3. The most likely explanation for this is the sediment depth. The surface evaporation discussed in Chapter 3 may have affected heavy metal behaviour by not only "buffering" the moisture content in the top 1 cm of the sediment, but also by bringing soluble heavy metals to the surface layer. Neither of these effects could have occurred in the current experiment and thus the changes are more defined, and the variability lower. However, after 48 days of oxidation, water/NH_4Cl-extractable concentrations (mg kg^{-1}) of Pb (53.4 ± 5.3 and 51.7 ± 3.3) and

Zn (1359 ± 264 and 1408 ± 35) were very similar for the experiment described in Chapter 3, and the current experiment respectively. Extractable Cd was ~20 % higher in the current experiment. Copper exhibited the least similar 48-day concentrations between the two experiments. Drying of the sediment in the current experiment exceeded that of the experiment described in Chapter 3 (Table 5.1, Table 3.3) and thus the sediment used in the current experiment is likely to have been more oxidised.

It was hypothesised in Chapter 3 that further oxidation may lead to an increase in mobile Cu. The current experiment provides evidence that this actually would indeed be the case (Fig. 5.4). However, the experiment described in Chapter 3 is perhaps a more realistic model of the canal site, where the sediment is more that 10 cm in depth.

5.4 Conclusions

The Woolston New Cut Canal sediment was shown to be highly phytotoxic. The maximum root elongation of both species was only ~ 65 % of root elongation in the control treatments. However, phytotoxicity also varied with the degree of oxidation of the sediment. Initially the sediment was anoxic and waterlogged and death of roots occurred, despite low available concentrations of heavy metals. Between 21 and 28-days of oxidation the sediment moisture content fell from 164 to 124 % and allowed root elongation. However, further drying and oxidation of the sediment caused a 0.9 unit drop in pH and large increases in heavy metal availability, which proved to be increasingly toxic to *B. napus*. The rooting behaviour of *L. multiflorum* made evaluation of phytotoxicity more difficult for this species In summary, the sediment was toxic to the seedlings of both species. The reason for the toxicity changed with time from anoxia to low pH and high metal availability.

As the sediment was maintained at field capacity (93 % moisture) throughout the experiment, better results could possibly be gained from an experiment such as this by using wetland plants as experimental species. These species would be more physiologically adapted to the low-oxygen conditions in the root zone and to the presence of sulphides in the sediment. Use of wetland plant species may therefore allow the determination of the proportion of toxicity that was due to heavy metals and the portion that was due to the anoxic, water-logged conditions. This may lead to a recommendation that wetland plant species be planted on the sediment after it is dredged, rather than dredging the sediment and planting it with terrestrial species which may not be able to survive the low-oxygen conditions. The use of terrestrial species in this experiment show that the newly dredged sediment is likely to be toxic to terrestrial plant species for several weeks after it is dredged and planting should not be carried out during this time.

CHAPTER 6

The Efficacy of Amendments in Counteracting Acidity, Decreasing Metal Lability and Reducing Phytotoxicity in Oxidised Canal Sediment.

6.1 Introduction

The results presented in Chapters 3 and 4 show that heavy metals tend to become more mobile in the WNCC sediment as it oxidises. The results presented in Chapter 5 show that this increase in metal availability, coupled with the decrease in pH, increased phytotoxicity of the sediment. The results presented in this Chapter examine the efficacy of selected ameliorants in reducing phytotoxicity and metal bioavailability and increasing pH in the fully oxidised sediment. The amendments tested were lime, red mud and di-potassium hydrogen phosphate.

6.1.1 Amendments

Estimates of available heavy metal concentrations are arguably a better measure of the toxic risk of a contaminated sediment than are total metal concentrations. Differing environmental conditions such as acidification or changes in redox state, may change the availability of sediment metals (Tessier *et al.*, 1979). Logically therefore, manipulation of sediment chemical and/or biological conditions can be used to reduce the availability of heavy metals. One form of manipulation, with an aim to reducing metal availability, is the addition of ameliorant materials here referred to as 'amendments'. Many such amendments have been tested including zeolites (Chlopecka *et al.*, 1997, Gworek, 1992, Gworek *et al.*, 1991a, Gworek *et al.*, 1991b), lime (Berti *et al.*, 1997, Bolton, 1975, Hamon *et al.*, 2002, Lombi *et al.*, 2003, Lombi *et al.*, 2002), beringite (Lombi *et al.*, 2003, Lombi *et al.*, 2002, Mench *et al.*, 1994, Vangronsveld *et al.*, 1995a, Vangronsveld *et al.*, 1995b), "red mud", a bauxite residue (Hamon *et al.*, 2002, Lombi *et al.*, 2003, Lombi *et al.*, 2002), K_2HPO_4 (Berti and Cunningham, 1997, Hamon *et al.*, 2002) and hydroxyapatite (Boisson *et al.*, 1999). Various other amendments have also been tested by these authors, including basic slags, hydrous

Mn oxides, steel shot (Mench *et al.*, 1994), gypsum, sulphur, organic carbon (Berti and Cunningham, 1997) and a kaolin amorphous derivative (Hamon *et al.*, 2002).

The amendments are all intended to reduce the concentration of potentially toxic metals in the soil solution. This can be achieved by increasing adsorption of metals, entrapment of metals in crystal lattices or precipitation of soluble metal species (Hamon *et al.*, 2002). The mode of action of the amendment affects the reversibility of the metal immobilisation and hence the long-term effectiveness of the treatment. If the amendment reduces metal availability by increasing the pH of the soil, then it is vulnerable to acidification, which is likely to increase metal availability to its original value (Hamon *et al.*, 2002). Ideally, to be effective in the long-term, an amendment will immobilise soluble metal species into non-labile forms which will then be more resistant to changes in environmental condition such as pH change.

In this study, the efficacy of lime, red mud and K_2HPO_4 as amendments to the Woolston New Cut Canal (WNCC) sediment was tested. Lime was chosen to increase the pH of the sediment and thus decrease heavy metal mobility. Red mud is a bauxite residue, created during the production of aluminium. It is composed mostly of aluminium hydroxides and may contain trace metal impurities. Red mud is typically alkaline with high salinity due to high sodium concentrations. Use of red mud as an ameliorant is expected to increase the pH of the sediment and also provide a high-surface area binding phase for heavy metals. Di-potassium hydrogen phosphate is used as an ameliorant to encourage the production of insoluble metal phosphates. The heavy metals would therefore be bound into the mineral structure and would be effectively immobilised. This form of immobilisation would be longer lasting than a simple increase in pH or the provision of an aluminium hydroxide binding phase carrying a pH dependant charge. Lime must be added with the K_2HPO_4 amendment to counteract the acidity of the HPO_4^- group, which will further increase the benefits of the amelioration. Red mud and K_2HPO_4 were chosen as ameliorants for the WNCC sediment as they were previously found to be the most effective amendments in contaminated soils by Lombi *et al.* (2002) and Hamon *et al.* (2002) respectively.

6.1.2 Diffusive Gradients in Thin Films

Diffusive Gradients in Thin Films (DGT) techniques allow the measurement of element flux in soil and aquatic systems. The procedure is explained in Zhang and Davison (1995) and Zhang *et al.* (1995). DGT has been used to measure elements in water systems (Zhang *et al.*, 1995), at sediment surfaces in aquatic systems (Zhang *et al.*, 1995, Zhang *et al.*, 2002) and in soils and sediments (Fones *et al.*, 2004, Koster *et al.*, 2005, Lombi *et al.*, 2002, Naylor *et al.*, 2004, Zhang *et al.*, 1995). Metals have been the object of many of these studies, though the technique has also been applied to the measurement of sulphide (Naylor *et al.*, 2004) and phosphorous (Zhang *et al.*, 1998). A representation of a DGT device and its function are shown in Figure 6.1. The device is typically constructed of a backing plate, an ion exchange gel layer, a diffusive polyacrylamide hydro gel layer, a filter layer (to prevent biological fouling of the gel) and a front plate with an exposure window. Different gel types are used to bind different species. Chelex gel is most commonly used for heavy metal uptake, AgI (silver iodide) gel has been used to bind sulphide (Naylor *et al.*, 2004), and ferrihydrite gel to bind phosphorous (Zhang *et al.*, 1998). DGT devices have been produced and deployed *in situ* to assess effective concentrations, resupply and flux of elements (Denny *et al.* 1999, Webb and Keough, 2002, Alfarro-De la Torre *et al.* 2000).

The DGT device allows calculation of flux of the ionic species of interest – in this case heavy metals in a soil/sediment system. The device is deployed face down on the surface of a saturated soil/sediment, ensuring that the membrane filter is in full contact with the soil. Pore water metals diffuse through the filter and through the gel layer to the Chelex resin gel binding layer, where they are immobilised, reducing the effective concentration to zero. This immobilisation creates a concentration gradient through the diffusive gel layer and causes local depletion of metals in the pore water surrounding the exposure window (Naylor *et al.*, 2004). This depletion is counteracted by a resupply with metal from the solid phase, which leads to the attainment of a pseudo steady-state. This state is maintained until the reservoir of solid phase metal is significantly depleted (Harper *et al.*, 1998). Metal flux is calculated per unit area, using Fick's First Law of diffusion, which states that the flux of a diffusing species is proportional to its concentration gradient. For a full explanation of the model and calculations used, see Harper *et al.* (1998). If the DGT-induced flux is

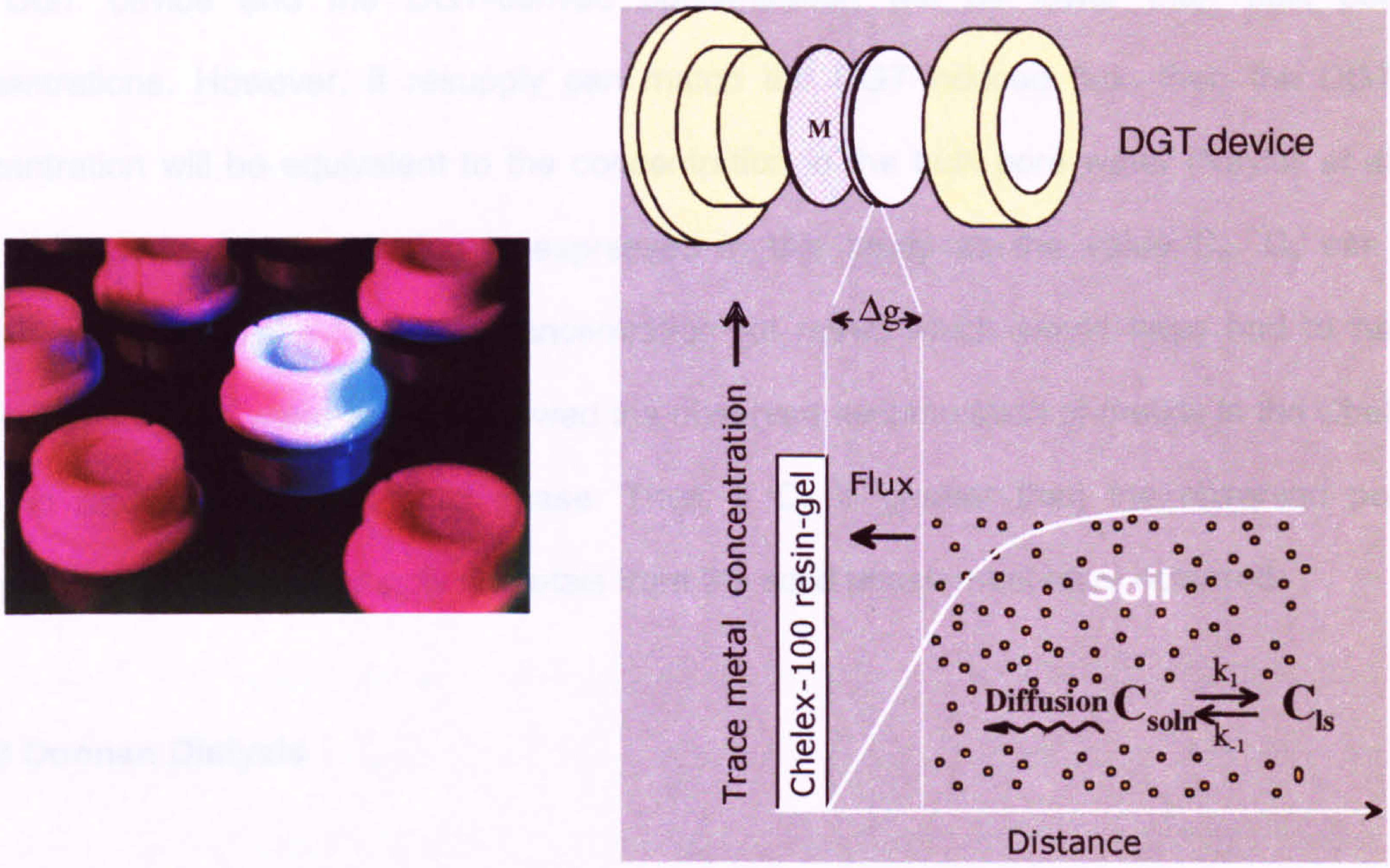


Figure 6.1. Assembled DGT devices, and a representation of how the DGT device works. Taken with permission from E. Lombi. C_{soln} is the concentration of metal in solution and C_{ls} is the concentration of metal on the labile solid phases and K_1 and K_{-1} represent the equilibria between the two. ΔG represents the thickness of the diffusive layers separating the soil solution from the Chelex resin (M).

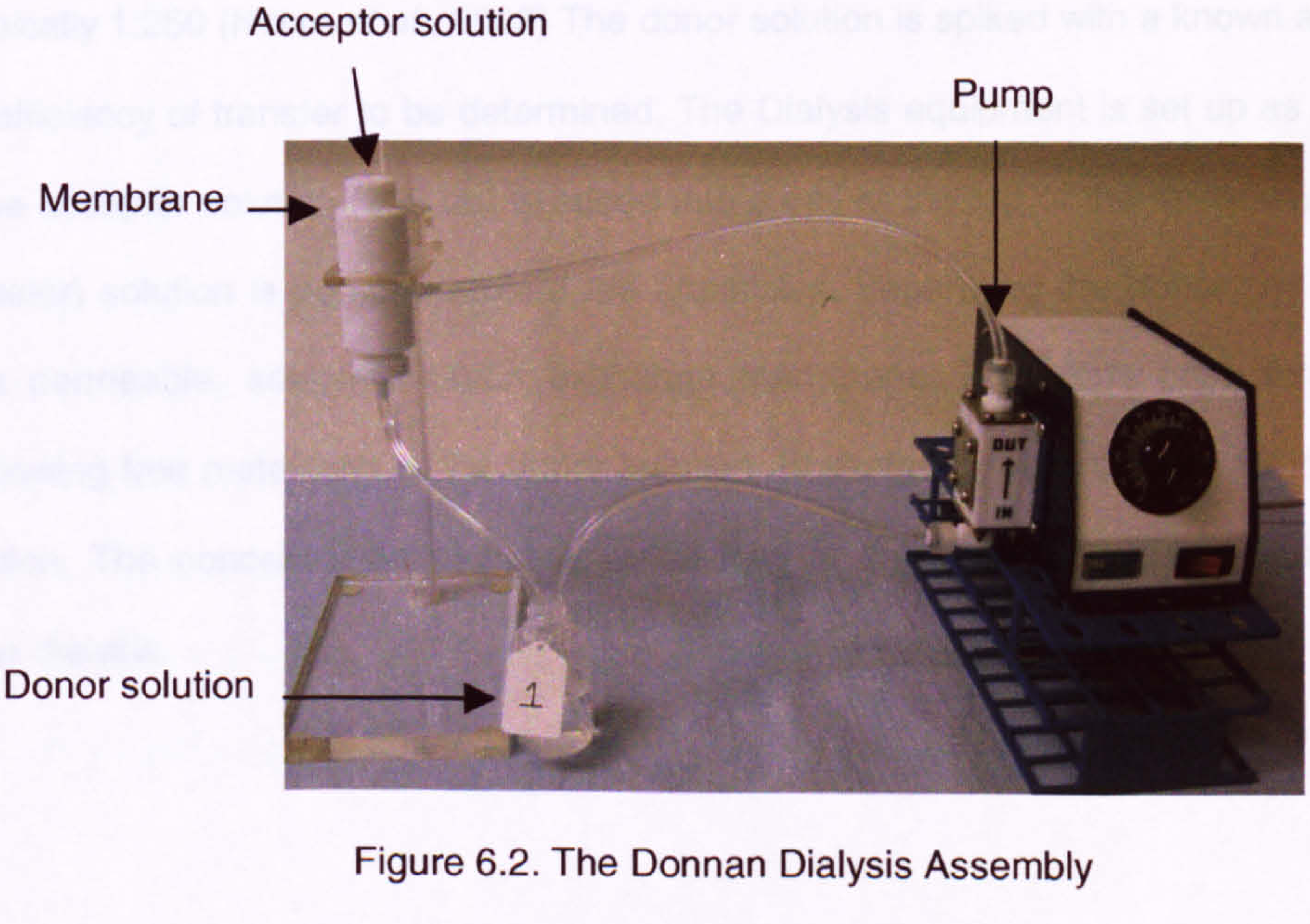


Figure 6.2. The Donnan Dialysis Assembly

greater than the rate of resupply, then pore water concentrations of metals will fall in the vicinity of the DGT device and the DGT-derived concentration will be lower than bulk pore water concentrations. However, if resupply can match the DGT-induced flux, then the DGT-derived concentration will be equivalent to the concentration in the bulk pore water (Naylor *et al.*, 2004). This DGT-derived concentration is expressed in this study as the value C_e. C_e can also be explained as being the "effective concentration" of metal which would have had to have been present in the pore water to have allowed the observed accumulation of metals in the Chelex resin, with no resupply from the solid phase. Thus, if C_e is greater than the observed pore water concentration (C), then resupply of metals from the solid phase must have occurred.

6.1.3 Donnan Dialysis

Donnan Dialysis is a technique which is used to determine free metal ion species in solution and was developed by Cox *et al.* (1984). This technique minimises errors usually associated with dialysis techniques such as large acceptor to donor solution ratios and has the additional advantages of being sensitive, multielemental and robust (Nolan *et al.*, 2003). During Donnan Dialysis, soil pore water is referred to as the "donor" solution. The "acceptor solution" is made to the same ionic strength as the pore water with strontium nitrate. The ratio of acceptor to donor solutions is typically 1:250 (Nolan *et al.*, 2003) The donor solution is spiked with a known amount of ^{22}Na to allow efficiency of transfer to be determined. The Dialysis equipment is set up as shown in Figure 6.2. The acceptor solution (200 μL) is added into a cell at the top of the apparatus and the donor (pore water) solution is pumped around the apparatus. Separating the donor and acceptor solutions is a permeable, selective cation exchange membrane. Free ions pass through the membrane, allowing free metal ions in the donor solution, to exchange with the free Sr ions in the acceptor solution. The concentration of heavy metal ions in the acceptor solution can then be measured after dialysis.

6.1.4 Aims

- To investigate the efficiency of lime, red mud and K_2HPO_4 as amendments to counteract the phytotoxicity of the sediment.
- To measure metal concentration in the pore waters of treated sediments.
- To evaluate metal immobilisation by the amendments, using DGT techniques.

6.1.5 Hypothesis

The addition of all amendments will decrease the pore water and free ion concentrations of heavy metals. Di-potassium hydrogen phosphate will be the most effective amendment as heavy metal ions will be immobilised within phosphate mineral structures. Red mud will be a more effective amendment that lime due to the added binding phase. Lime will be an effective amendment but will leave the sediment vulnerable to re-acidification. The flux of heavy metals will be reduced most by the di-potassium hydrogen phosphate amendment due to its immobilising effect.

6.2 Materials and Methods

6.2.1 Sediment

One hundred and sixty-six sediment cores were taken from along the length of the field site in December 2002 as described in Chapter 7. The sediment was air dried, ground and sieved to < 2 mm. Subsamples of approximately equal weight (~25 g) were taken from each of the 156 samples which had been taken from the planted plots. The 10 samples taken from the un-planted end section of the site were not included. The 156 subsamples were combined into one and thoroughly homogenised for use in the current experiment. They were sent to Australia by air.

Table 6.1. Proportions of amendments added to the WNCC sediment and the associated pH values (n = 3).

Treatment	Code	Final % red mud/ K_2HPO_4	Final % Lime	Final Total %	pH
No amendment	NA	-	-	0	3.6
1 % lime	L 1	-	1	1	4.4
2.5 % lime	L 2.5	-	2.5	2.5	6.2
5 % lime	L 5	-	4.8	4.8	7.2
1 % red mud	RM 1	1	-	1	3.7
2.5 % red mud	RM 2.5	2.4	-	2.4	3.9
5 % red mud	RM 5	4.8	-	4.8	4.3
5 % red mud, 5 % lime	RM 5 L5	4.6	4.6	9.2	7.2
1 % K_2HPO_4, 5 % lime	K 1 L5	1	4.7	5.7	6.9
2.5 % K_2HPO_4, 5 % lime	K 2.5 L5	2.3	4.6	6.9	7.2
5 % K_2HPO_4, 5 % lime	K 5 L5	4.6	4.6	9.2	6.8

6.2.2 Amendments

Amendments were added to the WNCC sediment in the proportions shown in Table 6.1. A 350 g sample of the homogenised air-dried WNCC sediment was weighed out for each treatment. Amendments were added as a w/w percentage of the 350 g of sediment to ensure comparable addition between amendments. Therefore the proportion of amendment in the final weight (Table 6.1) may appear to be less than the proportion stated, as addition of amendment increases the final weight of the bulk sediment. The sediment and amendment(s) were put into a bag and mixed thoroughly by hand. The bags were then sealed, ensuring the presence of an air space, and they were mixed on an end-over-end shaker for 48 h. Replicate pH measurements were taken on subsamples of the mixed sediment. To counteract the acidity of the HPO_4^- from K_2HPO_4 addition, lime ($CaCO_3$) was added to the K_2HPO_4 treatments to raise to the pH to approximately 7 (Hamon *et al.*, 2002), which constituted an addition of 5 % $CaCO_3$. The lime and the K_2HPO_4 were analytical grade reagents. The red mud originated in England.

6.2.3 Plant Growth Trial

Polyethylene pots with a top lip radius of 3 cm and a height of 10cm were used as growth vessels. The pots were tapered and decreased in diameter towards the bottom of the pot. For each treatment, 100 g of the sediment/amendment mix was weighed into the pot and a Rhizon pore water sampler (Rhizosphere Research Products, Wageningen, Holland) was inserted into each. All treatments were replicated three times. The sediment in the pots was made up to 50 % field capacity (FC) with deionised water, and left to equilibrate at room temperature for 5 d. The pots were then made up to 100 % FC and left to equilibrate for a further 24 h. Pore water was then extracted using the Rhizon samplers and pore water from the three replicate treatments was combined to provide sufficient volume for Donnan dialysis. Pore water pH was measured and total metal analysis was carried out by ICP-MS. Free ion activities of Cd, Cu, Ni, Pb and Zn were determined by Donnan Dialysis on the 5 % amendment treatments only due to the cost of this technique. The cost of the technique is high due to the dialysis taking place in a clean-room environment.

Rhizon pore water samplers have several advantages over other techniques such as centrifugation. Centrifugation of samples to extract the pore water can lead to alterations of the pH of the sample. This is not the case with a Rhizon sampler. The sampler also filters the pore water as it extracts it so that the pore water is ready for analysis or use as soon as it is extracted. As it is *in situ* the sampler also allows multiple samples of pore water to be taken during an experiment and does not damage any plants which are growing in it.

Pots were returned to 50 % FC with deionised water and 10mL of Ruakura nutrient solution. Twenty seeds of *Lolium multiflorum* (concorde ryegrass) were then planted in each. The pots were transferred to a controlled environment room that maintained a temperature of 20 °C, and a 16-hour photoperiod. The seeds were allowed to germinate and grow for 42 days before the aerial parts of the plants were harvested. Moisture content of the soil was maintained by weight with deionised water over the growth period. Observations of plant health and growth were taken throughout the growth period. After harvest, the pots were wetted up to 100 % FC and pore waters were again taken. Harvested plant material was dried at 40 °C.

6.2.4 Total Plant Metal Uptake

For analysis of metal concentrations in the ryegrass, 0.5 g of oven-dry plant material was digested in nitric acid using a hotplate method (Zarcinas and Cartwright, 1983, Zarcinas *et al.*, 1983). Where 0.5 g was not available, total mass of grass was digested. Metal analysis was carried out by ICP-MS and GF-AAS.

6.2.5 Red Mud

The red mud used had a pH of 10.5 according to Lombi *et al.* (2002), but the pH of the red mud-amended canal sediment was not as high as had been expected (Table 6.1). In this experiment the red mud treated sediments were not leached to reduce the salinity, in agreement with Lombi *et al.* (2002) but in contrast to Hamon *et al.* (2002). However, it was postulated that the red mud may have previously been washed, as the sample used had been labelled simply as "red mud" whereas another sample of the same material was labelled as "unwashed". Washing reduces the salinity of the red mud and may also have led to loss of some of its basic properties. Thus a sample of the red mud which was labelled as "unwashed" was added at the 5 % level to a new sediment sample in the same manner as described above. The pH of this mixture was tested and found to be no different to the pH of the original 5 % red mud treatment. It is therefore very unlikely that the red mud originally used had been washed. To raise the pH of the new 5 % red mud amendment, 5 % lime was added as had been done to the K_2HPO_4 treatments. Due to the late starting of this final treatment, pore water samples were not taken. Instead the sediment was wetted to 50 % FC and left to equilibrate for 5 d before planting. The *Lolium* was allowed to grow for the same time period as in the other treatments (42 d) before harvest. Total metal analysis of the red mud is given in Table 6.2.

6.2.6 Donnan Dialysis

Donnan Dialysis was carried out on the 5 % amendment treatments only, with the exception of the 5 % red mud, 5 % lime treatment. The ionic strength of the pore water was determined immediately after it was extracted. An "acceptor" solution was then made up to an equal ionic strength with strontium nitrate. The pore water was dialysed for 2 h and then ion concentrations in both the pore water and the acceptor solution were measured by ICP-MS and GF-AAS. From these data, the percentage of metal in the pore water that was present as free ions was determined.

6.2.7 DGT

Standard cylindrical DGT units (Fig. 6.1) were obtained from DGT Research Ltd. For details of production of the gels and the standard DGT protocol, see the review by Davison *et al.* (2000). Assembly of the device was carried out in a clean room in a 100 laminar flow cabinet. The units were acid washed and thoroughly rinsed with MilliQ water before assembly. Assembled devices were stored in a clean plastic bag in a fridge until needed, and were allowed to reach room temperature before deployment.

Table 6.2 Total metal content of the red mud extracted by a hot aqua regia digest. (From Lombi *et al.,* 2002).

Metal	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**
mg kg^{-1}	8.2	449	83.9	4395	246	175	151

Separate 20 g samples of the amended soil were weighed in duplicate into small polyethylene pots, wetted up to 100 % FC and left to equilibrate for 24 hours. The length of time for which DGT devices were deployed differed between treatments and was calculated from the pore water Zn concentrations. Higher concentrations gave a lower deployment time so as to avoid the possibility of the exchange resin becoming saturated. Deployment times ranged from 2.2 to 24 hours and samples were kept in a controlled temperature room at 21 °C during this time.

After the appropriate time, the devices were removed from contact with the soil and rinsed with MilliQ water to remove residual soil particles. Devices were disassembled and the Chelex resin gel was carefully removed with acid-washed plastic tweezers, and transferred to 2 mL Epindorff tubes. Gels were eluted in 1 mL of 0.1 M HNO_3 for at least 48 h. For analysis of eluted metals, 700 µL of the acid was removed and made up to 7 mL in 1 % HNO_3. Metal analysis was carried out by ICP-MS.

6.2.8 Statistical Analysis

Statistical analysis was carried out using the statistical package SPSS. Kruskall-Wallis analysis was used for comparing > 2 variables. For comparison of 2 variables, Mann-Whitney U tests were employed. The p-values quoted in this chapter are derived from these tests.

6.3 Results

6.3.1 pH

The addition of lime to the WNCC sediment led to an increase in pH which varied with the percentage level of addition. The highest pH (pH 7.2) was attained when 5 % lime was added to the sediment. Addition of 1 % lime led to an increase of pH to only 4.4. All K_2HPO_4-treated sediment samples had near-neutral pH due to the associated lime addition. Lombi *et al.* (2002) found that addition of red mud (presumably at the 2 % level) to their soils led to an increase in pH greater than observed upon addition of the same dose of either lime or beringite. In the present experiment, addition of red mud had very little effect on the pH of the sediment, even at the 5 % level, and performed poorly in comparison to the lime treatment (Table 6.1). Few treatments showed an alteration in pH over the growth period though the 2.5 % lime treatment decreased from pH 6.2 to pH 5.9 over this time (data not shown). Thus reacidification may be a future risk for this site.

6.3.2 Plant Biomass and Uptake

The average biomass of the *Lolium multiflorum* harvested per pot is shown for each treatment in Figure 6.3. From these data it is evident that the unamended sediment is very phytotoxic and resulted in minimal *Lolium* growth. All of the amendments added did increase the biomass production, though red mud had the least effect (Fig 6.3). The addition of 1 % lime also had only a small effect on biomass production. The addition of 5 % lime had little more effect on biomass that did addition of 2.5 % lime. There was no significant difference in biomass production between the 2.5 % lime, 5 % lime, 5 % red mud (with 5 % lime), 1% K_2HPO_4 (with 5 % lime) and 2.5 % K_2HPO_4 (with 5 % lime) treatments ($p > 0.05$). The addition of 5 % K_2HPO_4 (with 5 % lime) however, did show a large increase in biomass, perhaps in part due to the high concentrations of phosphate that were introduced by this amendment. However, amending the WNCC site with 10 % by weight of an amendment mixture is not practical or economical. The biomass data would suggest lime addition at the 2.5 % level is the most effective and efficient amendment, but this would leave the sediment vulnerable to acidification (Hamon *et al.*, 2002) and in a longer term trial may not prove to be viable.

Observations taken during the growth period are given in Appendix 5. Values such as the percentage of plants displaying a certain feature are estimates and the descriptions of "growth" are relative to the other treatments, though height of the tallest plant was measured in each pot. The toxicity symptoms observed during growth (Appendix 5) suggest that the reason for the poor growth is metal toxicity rather than simply the pH differences. This is further supported by the plant heavy metal contents. Figures 6.3 and 6.4 show that metal concentrations in the plant tissues

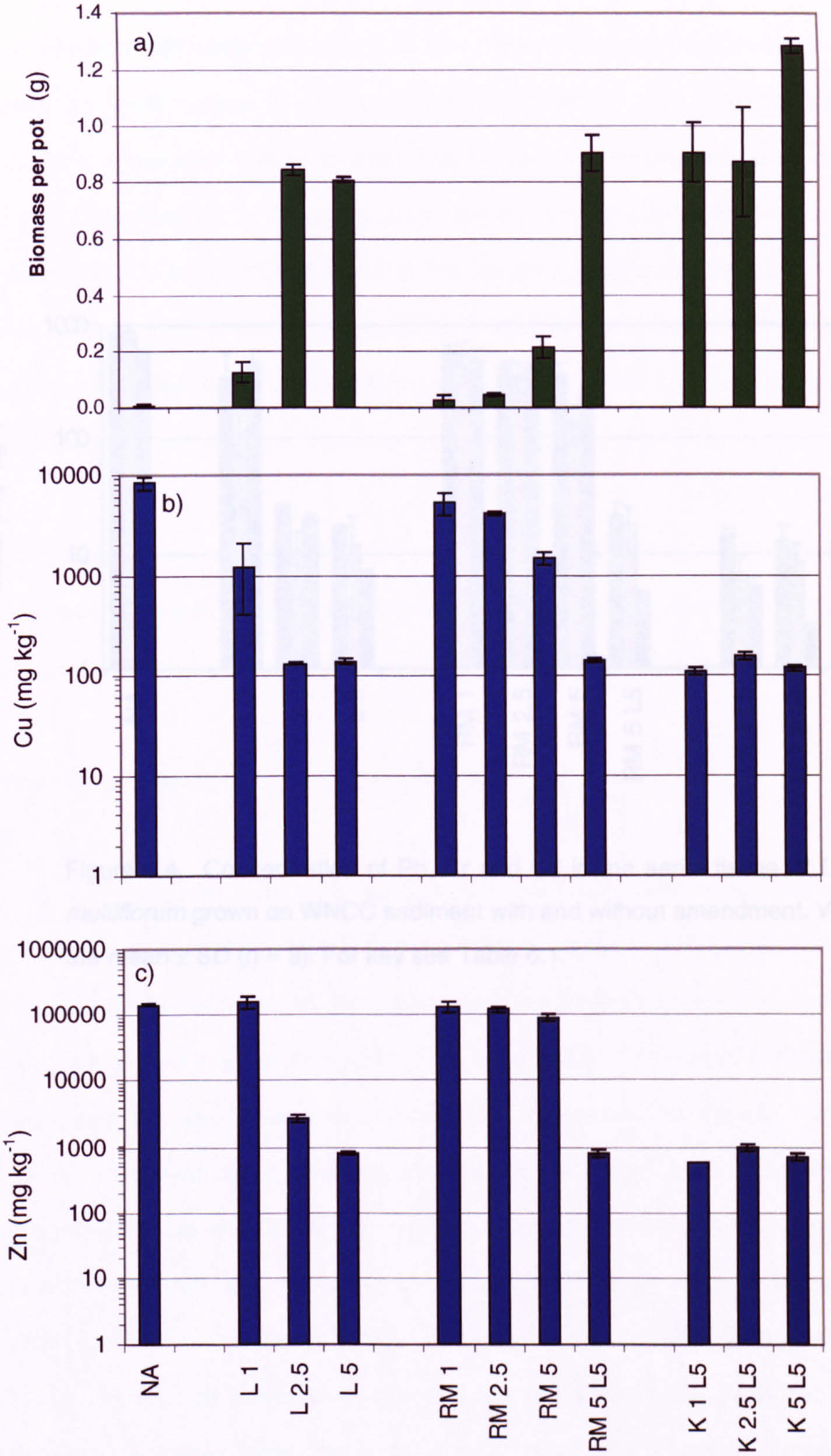


Figure 6. 3. a) Biomass harvested per pot b) Cu and c) Zn uptake by *Lolium multiflorum* in WNCC with and without amendment. Values are mean ± SD (n = 3). For key see Table 6.1.

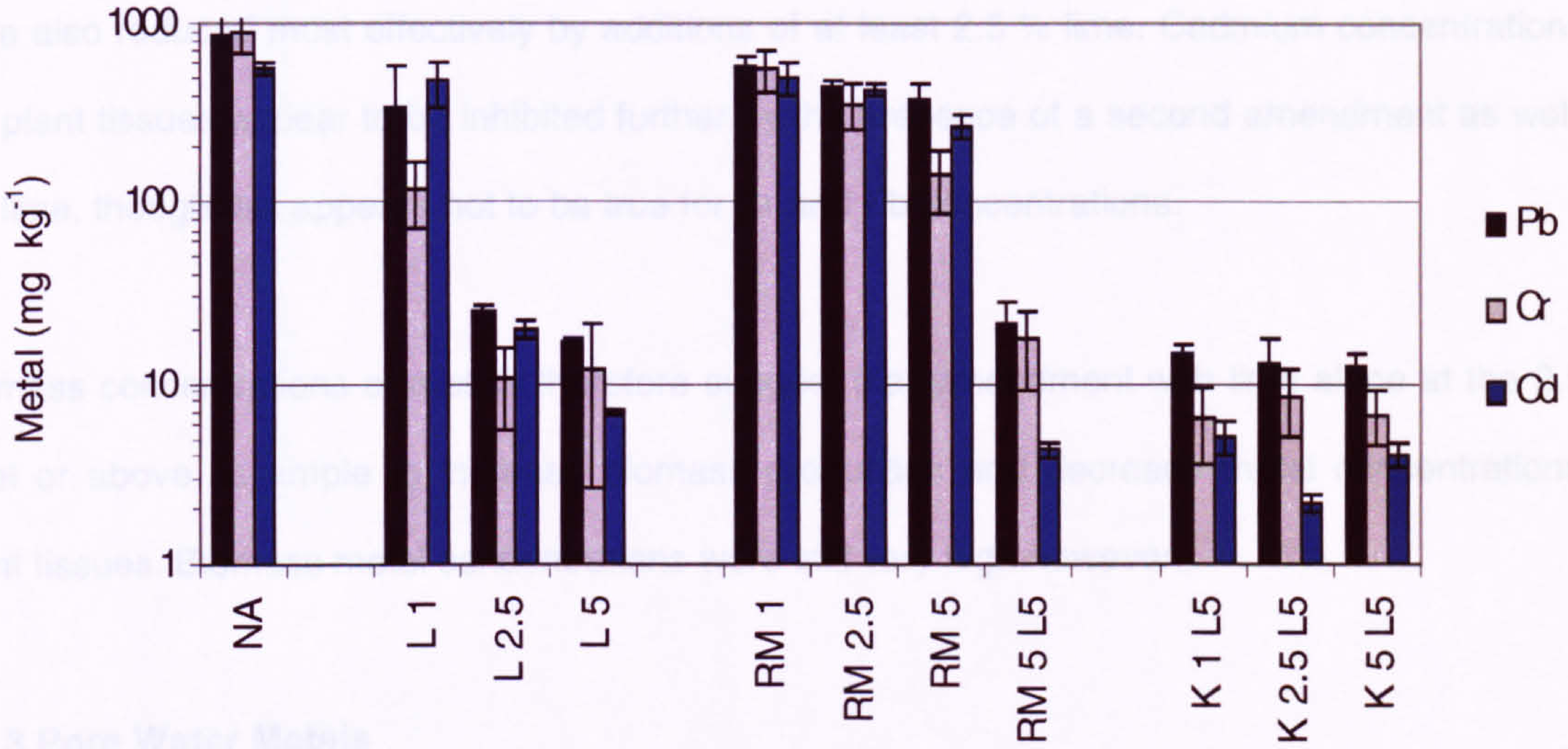


Figure 6.4. Concentration of Pb, Cr and Cd in the aerial tissue *of Lolium multiflorum* grown on WNCC sediment with and without amendment. Values are mean ± SD (n = 3). For key see Table 6.1.

varied greatly between treatments. The 1 % lime treatment and the red mud-only treatments, had little effect on plant uptake of heavy metals compared to the untreated sediment. Copper concentrations in the plant tissues differed little between the treatments containing 2.5 % lime or above, and concentrations of Zn in the plant tissues differed little between all of the treatments which contained 5 % lime. Figure 6.4 shows that Pb, Cr and Cd concentrations in the plant tissues were also reduced most effectively by additions of at least 2.5 % lime. Cadmium concentrations in the plant tissues appear to be inhibited further by the presence of a second amendment as well as the lime, though this appears not to be true for Cr and Pb concentrations.

Biomass concentrations of metals therefore suggest that amendment with lime alone at the 2.5 % level or above, is ample to increase biomass production and decrease metal concentrations in plant tissues. Biomass metal concentrations were still very high however.

6.3.3 Pore Water Metals

Though amendments may have an immediate effect on metal levels in pore waters, it is important in site reclamation that the effect of the amendment lasts in the longer term. In the WNCC sediment, growth of ryegrass on the unamended sediment appeared to increase pore water concentrations of all of the metals studied (Figs. 6.5 and 6.6). The addition of amendments caused an initial decrease in pore water metals compared to the unamended sample, except in the case of Pb in the red mud treatments. However, after the growth period, the 1 % red mud treatment had mobilised more Pb, Cd and Zn than the addition of no amendment. This is again in disagreement with the work of Hamon *et al.* (2002) and Lombi *et al.* (2002), who report both an increase in pH upon addition of red mud, and the fixation of metals in a non-labile, partially acid resistant form. This discrepancy may be explained by the low initial pH of the WNCC sediment. It is possible that the formation of these non-labile phases requires a near-neutral pH and thus they are not formed in the WNCC; hence the extra benefit they would otherwise confer, is lost.

Treatments containing 2.5 % lime or more, effectively and very significantly reduced pore water levels of all metals studied (Figs. 6.5 and 6.6). Table 6.3 shows the percentages of the pore water

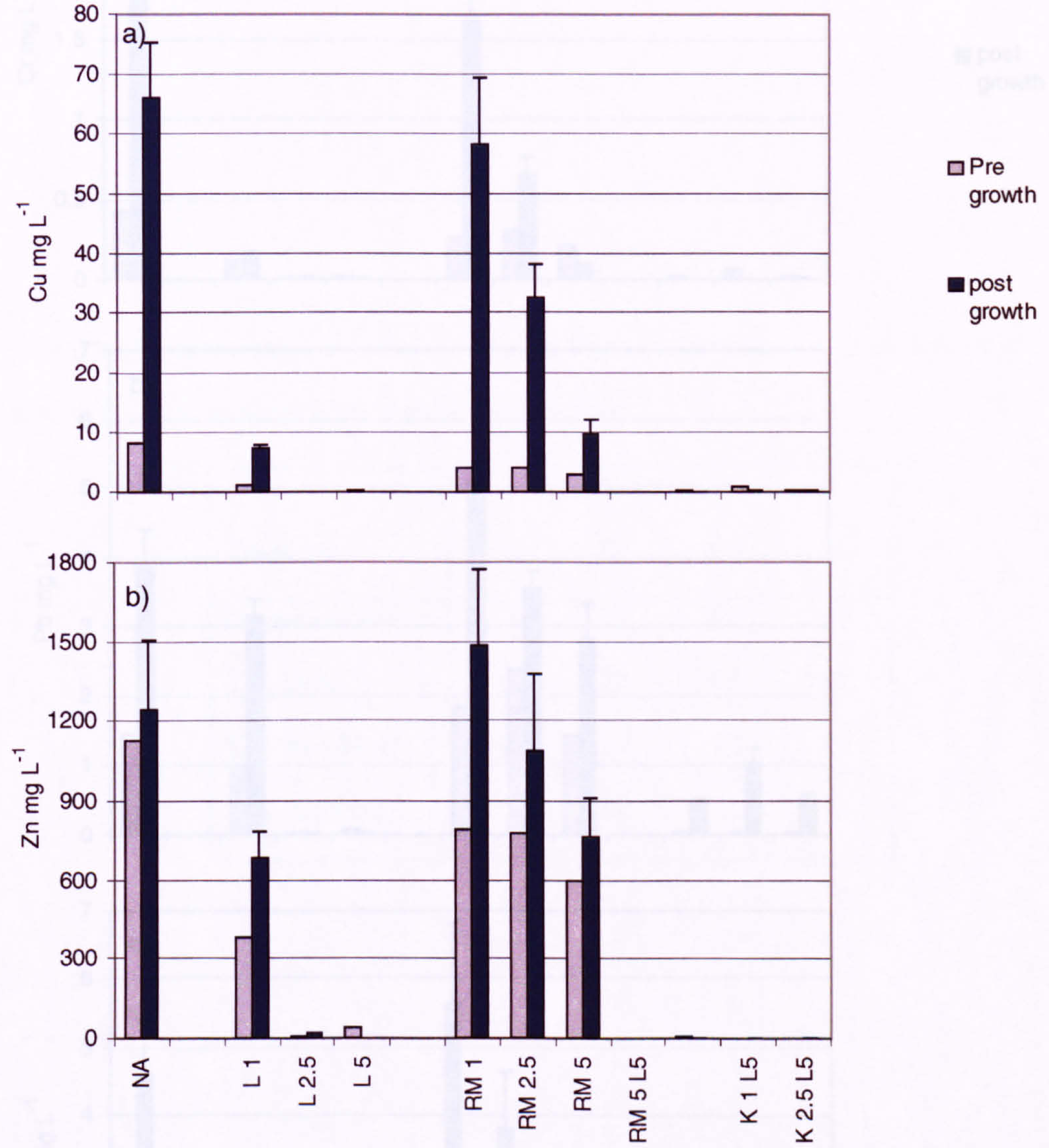


Figure 6.5. Pore water concentrations of a) Cu and b) Zn before and after growth of *Lolium multiflorum* in amended and unamended WNCC sediment. Pre-growth pore waters were pooled and represent absolute values, post-growth values represent mean ± SD (n= 3). For key see Table 6.1.

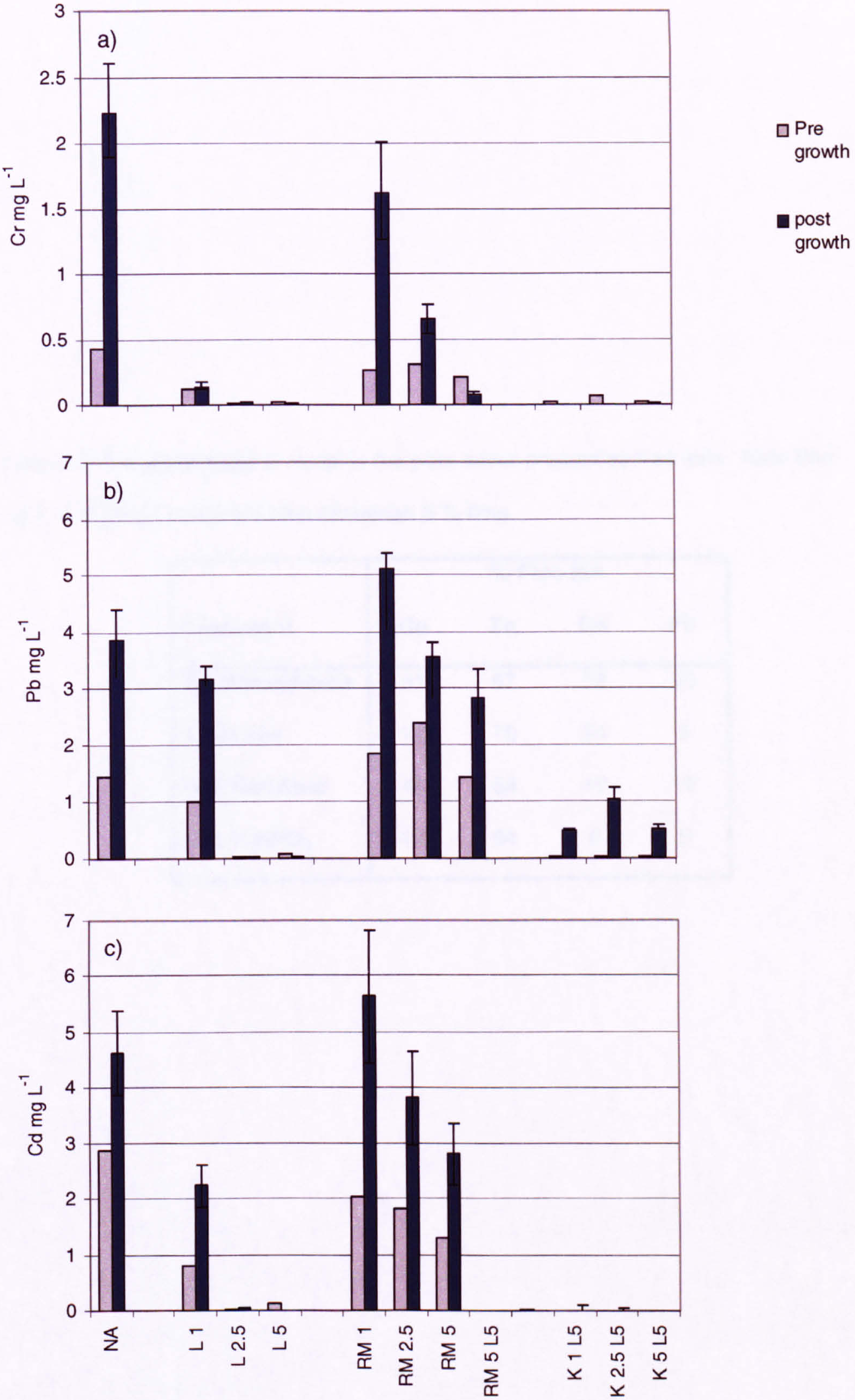


Figure 6.6. Pore water concentrations of a) Cr b) Pb and c) Cd before and after growth of *Lolium multiflorum* in amended and unamended WNCC sediment. Pre-growth pore waters were pooled and represent absolute values, post-growth values represent mean ± SD, (n = 3).For key see Table 6.1

Table 6.3 The percentage of metal in the pore water present as free ions. Note that the 5 % K_2HPO_4 treatment also contained 5 % lime.

	% Free ion			
Treatment	**Cu**	**Zn**	**Cd**	**Pb**
No Amendment	51	57	59	20
5 % Lime	1.6	78	54	8
5 % Red Mud	46	64	48	19
5 % K_2HPO_4	1.6	64	0	0

metals that were present as free ions, as determined by Donnan Dialysis. The percentage of Cu present as free ions was reduced very effectively by the addition of 5 % lime and the 5 % K_2HPO_4 (with 5 % lime) treatments. A small reduction was evident with the addition of the 5 % red mud treatment. The percentage of Zn which was present as the free ion was increased by addition of all amendments. The addition of lime alone led to the largest increase. The addition of the 5 % lime amendment and the 5 % red mud amendment had a small effect on the percentage of pore water metal which was present as the free ion. The addition of the 5 % K_2HPO_4 (with 5 % lime) treatment however, entirely removed the free ion component of pore water Cd. The same was true for Pb. This is likely to have been due to the reaction of these two metals with the phosphate group, forming insoluble metal phosphates. The addition of red mud had little effect on the percentage of Pb present as free ions, though addition of lime alone reduced it by a little more than a half. Thus, though the differences in pore water concentrations of heavy metals between the 5 % lime and 5 % K_2HPO_4 (with 5 % lime) treatments may not be obvious from the Figures 6.5 and 6.6, the extra effect of the K_2HPO_4 in further reducing the free ion concentrations in solution is clear from these data. This may reduce the toxicity of the pore water and help to explain the better *Lolium* growth in the 5 % K_2HPO_4 (with 5 % lime treatment). Despite the negligible concentrations of metals in the pore water in the high lime containing treatments, plant uptake of metals was still significant in all cases. One reason for this could be that, though pore water concentrations were at a very low level, resupply from the solid phase to maintain those concentrations may have allowed uptake of significant concentrations of metals over the growth period.

6.3.4 DGT

To test the resupply of depleted pore water metals from solid phase sources, DGT techniques were employed. The results of the analysis showed that resupply from the solid phase was occurring (i.e. $C_e > C$) for all metals studied, with all amendments, with the following exceptions: Zn: 5 % lime, 1 % Red mud and 2.5 % Red mud, and Cd: 5 % lime (data not shown). The DGT devices were not redeployed after plant growth to find out if these dynamics had changed. If they had not and the metals had been effectively immobilised for the duration of the growth period then pore water metal concentrations could not have risen during this time. Figures 6.5 and 6.6 show that Zn and Cd may

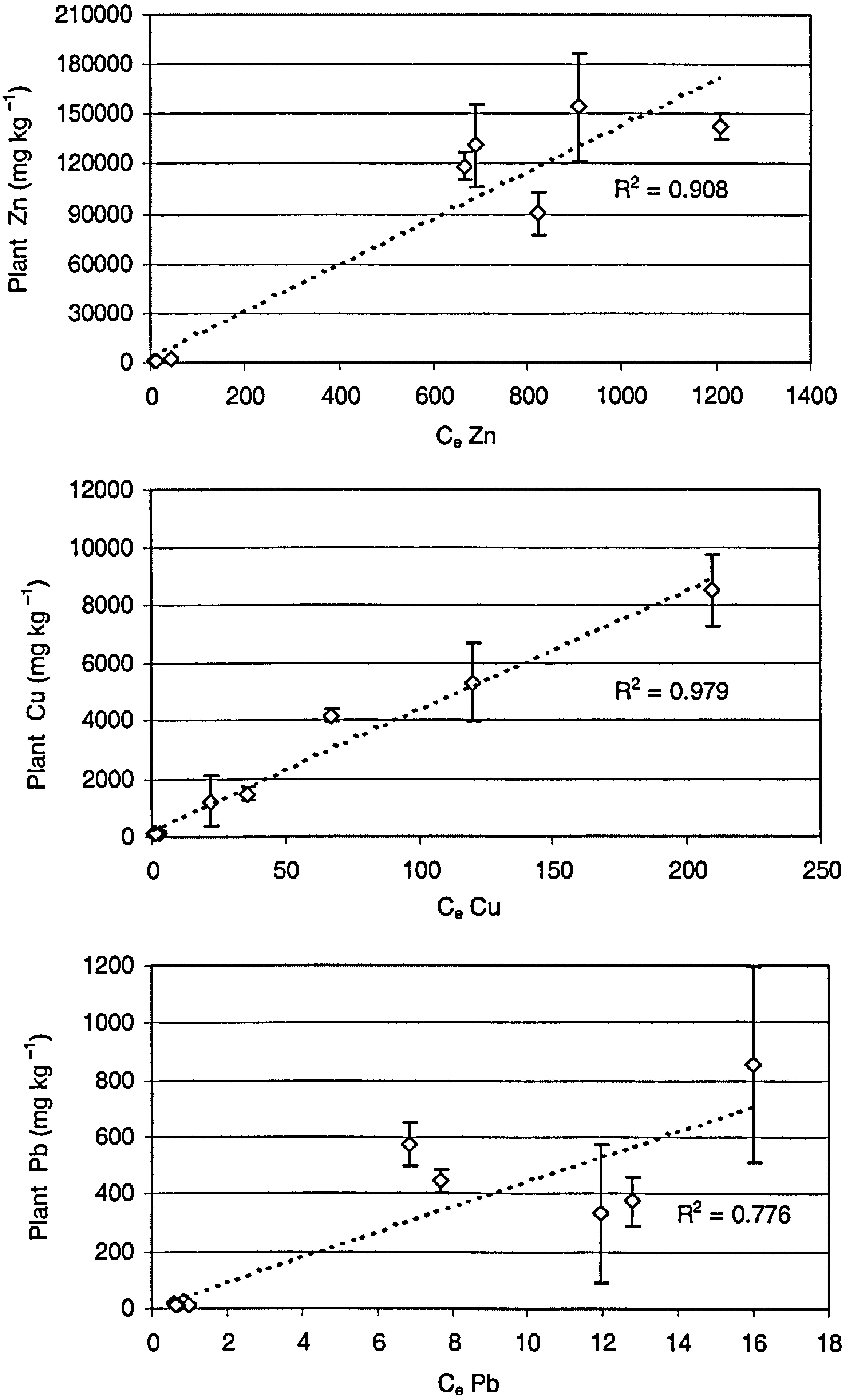


Figure 6.7. The relationship between equilibrium concetration (C_e) and plant uptake of Zn, Cu and Pb for all WNCC sediment treatments. Values are mean ± SD (n = 3)

Table 6.4. Metal flux (ng cm^{-2} s^{-1}) from solid phases into soil solution before growth of *Lolium multiflorum*. Note the different orders of magnitude of the data. Values are mean (SD) (n = 2).

	Metal flux ng cm^{-2} s^{-1}				
Treatment	**Cd (x10^{-3})**	**Cr (x10^{-3})**	**Cu (x10^{-2})**	**Pb (x10^{-2})**	**Zn (x10^{-2})**
No Amendment	29 (6.7)	30 (6.7)	151 (3.4)	15 (0.85)	850 (130)
1 % Lime	11 (2.6)	3.5 (2.6)	11.4 (5.6)	7.6 (2.9)	419 (76)
2.5 % Lime	1.5 (3.5)	3.4 (3.5)	0.57 (0.28)	0.77 (0.63)	30 (4.3)
5 % Lime	0.47 (0.84)	2.9 (0.84)	0.53 (0.08)	0.53 (0.14)	5.67 (1.0)
1 % Red Mud	16 (4.9)	14 (4.9)	86.5 (15)	6.4 (1.2)	484 (61)
2.5 % Red Mud	15 (7.8)	8.3 (7.8)	48.6 (17)	7.1 (3.6)	469 (130)
5 % Red Mud	15 (15)	20 (15)	25.7 (3.0)	12 (3.8)	579 (110)
1 % K_2HPO_4 + 5 % Lime	0.61 (1.2)	4.8 (1.2)	0.78 (0.06)	0.93 (0.20)	9.54 (0.95)
2.5 % K_2HPO_4 + 5 % Lime	0.50 (3.4)	3.5 (3.4)	2.18 (0.34)	0.61 (0.59)	5.27 (1.6)
5 % K_2HPO_4 + 5 % Lime	0.67 (2.7)	3.1 (2.7)	1.02 (0.33)	0.59 (0.51)	9.49 (2.9)

have been effectively immobilised throughout growth by the 5 % lime treatment as suggested by the DGT data, as metal in the pore water decreased to negligible concentrations over the growth period. However, this is not true for the red mud treatments, suggesting that the immobilisation of metals in this sediment by red mud addition was only temporary. The high uptake of Zn and Cd by *Lolium* is evidence that the low pore water concentrations are in fact being maintained by resupply from the solid phase. DGT devices should therefore be deployed throughout an experiment of this nature, or at least at the beginning and end, in order to quantify changes in amendment effectiveness over the experimental period.

Figure 6.7 shows C_e (the effective concentration) vs. plant uptake. The distribution of the data points on the Zn graph is typical also for Cd and thus the Cd data is not shown. The Cd R^2 value was 0.905. Uptake of Cd, Zn and Cu showed a good correlation with the DGT-derived C_e values. This means that the effective concentration (C_e) of these three metals in solution (maintained by resupply from the solid phase) was controlling the concentration of the metals in the plant tissues. This was likely to be via controlling phytoavailability of the metals. Due to the high R^2 values, in future experiments it may be possible to predict C_e from *Lolium multiflorum* uptake of metals with some accuracy. This would be especially true for Cu, for which plant tissue concentrations appeared to be almost completely controlled by the effective pore water concentration of Cu. Lead showed a poorer correlation and these data could not be used to accurately predict either C_e from plant uptake or vice versa. The differing chemistry of the elements is likely to be the cause of this difference in the predictive capabilities of DGT in this case.

The DGT data were used to calculate metal flux and the results are shown in Table 6.4. In the un-amended sediment the rate of flux of the metals quantified were in the following order (decreasing) Zn>Cu>Pb>Cr = Cd. The addition of 1 % lime, 1 %, 2.5 % and 5 % red mud all reduced the flux of Zn by around 50 %. This means that resupply of solution concentrations of Zn, when depleted, would be replenished from the labile solid phases at half the rate that they would do in the unamended sediment. Addition of 2.5 % and 5 % lime reduced the flux by Zn by one and two orders of magnitude respectively. The addition of the K_2HPO_4 (with 5 % lime) did not reduce the flux of metals any more than did addition of the 5 % lime alone. Values were in fact slightly increased, most probably due to the reaction of some of the lime with the HPO_4^-. Copper flux was decreased most effectively by addition of ≥ 2.5 % of lime. Addition of K_2HPO_4 with the 5 % lime increased flux of Cu, possibly for the reason stated above. Red mud was the least effective amendment, though flux of Cu did decrease with increasing percentage additions of red mud. The flux of Pb from the labile solid phases was most reduced by the addition of ≥ 2.5 % lime or 2.5 % K_2HPO_4 (with 5 % lime). Red mud was again the least effective amendment at reducing Pb flux and increasing additions of red mud appeared to increase Pb flux with respect to the 1 % addition. It is probable that this effect was due to the mobilisation of some of the Pb within the red mud amendment (Table 6.2), under the low pH conditions. The flux of Cr was reduced by a factor of 10 by the addition of lime, though the level of addition had no significant effect on the flux i.e. the flux was no lower after addition of 5 % lime that after addition of 1 % lime. The same was true of the K_2HPO_4 amendment. Red mud was once again the least effective amendment. Red mud was also the least effective amendment at reducing the flux of Cd from the labile solid phases into solution. Lime additions at ≥ 2.5 % were again the most effective and addition of the K_2HPO_4 with the 5 % lime conferred no significant benefit in terms of flux reduction.

6.4 Conclusions

All amendments reduced the flux of metals from the solid phase into solution compared to the unamended sediment. The 5 % lime amendment appeared to do this most effectively. However, it should be remembered that the DGT data is a snapshot and the situation may change over time or with changing condition such as reacidification of the sediment. The K_2HPO_4 was the next most effective amendment, but this should be investigated further to determine whether this is simply due to the lime added with this amendment rather than the effect of the amendment itself. The metal flux in the WNCC sediment, both amended and unamended, was much higher than that reported by Lombi *et al.* (2002) for a French soil with comparable levels of Zn, Pb and Cd contamination. Lombi found no significant difference in metal flux between soil treated with lime and red mud, even in the case of Zn. In this study, there were obvious and sometimes very large differences in the reduction in metal flux conferred by the amendments, especially in the case of Zn (Table 6.4). However, all of the amendments reduced metal flux with respect to the unamended control and overall, lime at the 5 % amendment addition level appeared to have the most beneficial effect.

All treatments resulted in a less phytotoxic sediment although the combined 5 % K_2HPO_4 and 5 % lime amendment resulted in the highest biomass production. However, pore water concentrations and plant uptake for this treatment were similar to those for the 5 % lime only treatment. Addition of amendments at the 10 % levels are not economically viable at this site, thus further work is needed to determine the efficacy of the K_2HPO_4 amendment with very low percentage levels of lime addition complementing it. The red mud did not perform as expected, possibly due to the acidic nature of the WNCC sediment, and it has no value for use as an amendment of this sediment. Lime at the 2.5 % level or above, proved to be the most effective agent at reducing phytotoxicity of the sediment. However, reacidification of the canal sediment with time is likely to completely undo the effects of the lime. It can be concluded that, for the amendments used in this study to be effective on a field scale, large percentage additions may be required. This may not be economically viable on a site with so little economic value. Further testing of the K_2HPO_4 amendment would be needed on a laboratory scale before any field testing was carried out, to determine its longevity and if it has any benefits above the use of lime alone. Small scale field trials would also be necessary to determine the behaviour of the amendments in the sediment under normal environmental conditions.

CHAPTER 7

Phytoremediation of the Woolston New Cut Canal Sediment: An Evaluation of the Efficacy of the Phytoremediation Field Trial

7.1 Introduction

The experiments described in Chapters 3 to 6 were carried out under controlled laboratory conditions. However, the planting platform sediment at the Woolston New Cut Canal (WNCC) site was subject to environmental conditions for the duration of the current project. This will undoubtedly have altered the behaviour of the pollutants in the system with respect to the behaviour described in previous chapters. The results presented in this chapter examine the changes in metal availability and total metal concentrations in the planting platform sediment. Growth of, and heavy metal uptake in, experimental trees were also examined. Data for the degradation of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH) are also presented. Very few studies examine phytoremediation of sediment naturally contaminated with both heavy metals and organic pollutants in long-term field trials. A limited number of studies have focussed on river sediments, generally with lower pollution levels than the present study. However, all of these sediments have had appreciable $CaCO_3$ contents and have resisted acidification upon oxidation. To the author's knowledge this is the first field trial to examine the phytoremediation of a sediment contaminated with both organic and heavy metal pollutants, using SRC (short rotation coppice) trees, on a naturally acidifying sediment, over a 3-year period.

7.1.1 Phytoremediation of Aquatic Sediments

Aquatic sediments from estuaries, rivers and canals are regularly dredged to allow continued navigation along the waterways. Contamination in these sediments is common. These three different types of sediment may have different structural and chemical properties as well as varying salinity. On top of this, each individual sediment varies in the type and degree of contamination it contains. Phytoremediation of aquatic sediments using *Salix* clones has been undertaken elsewhere. Vervaeke *et al.*, (2001) report a technique for planting *Salix* on wet, dredged sediments called the SALIMAT technique. This technique is promising for the phytoremediation of large lagoons, with above ground biomass production after 4 years reported to be 55.7 DM t ha^{-1}, equivalent to 13.9 t ha^{-1} y^{-1}. Pulford and Watson (2003) report yields of 10 – 15 DM t ha^{-1} y^{-1} as being normal for *Salix*. However, if tissue concentrations of heavy metals are low, phytoremediation of a contaminated site to below guideline levels may take decades, even with reasonable biomass production (Pulford and Watson, 2003). If the phytotoxicity of the substrate is too high, biomass production will be reduced. Vandecasteele *et al.* (2005) found that the hydrological regime to which *Salix cinerea* was exposed when planted on dredged sediment, affected metal uptake into foliar tissues. At the end of the growing season, the highest foliar concentrations of Zn and Cd were reported for *S. cinerea* growing in plots which had initially been flooded, but had emerged in the early part of the growing season. Foliar heavy metal concentrations were also monitored throughout the growing season. Cadmium, Fe, S, Mn and Zn concentrations in *Salix cinerea* leaves all increased throughout the growing season, reaching, with the exception of sulphur, maximum concentrations at the end of the growing season. Conversely, Cu concentration was reduced at the end of the growing season compared to the beginning. The rate of Cd and Zn uptake also increased through the growing season (Vandecasteele *et al.*, 2005).

Oil is ubiquitous in aquatic sediments (Jonker *et al.*, 2003). The WNCC site is contaminated with organic pollutants, namely TPH (total petroleum hydrocarbons) and PAH (polycyclic aromatic hydrocarbons). The presence of oil in a sediment may affect the partitioning and bioavailability of hydrophobic organic chemicals. The bioavailability of PAH may be enhanced at low oil concentrations and decreased at high oil concentrations (Jonker *et al.*, 2003). Organic contaminants which are bioavailable can be degraded by biota. Plant roots form a rhizosphere which can increase microbial activity in general, and also specifically the activity of those bacteria which are able to degrade petroleum (Kirk *et al.*, 2005). However, Vervaeke *et al.* (2003) reported a greater reduction in the concentration of PAH in fallow as opposed to planted plots. They hypothesised that this was due to the shrinkage and cracking of the fallow sediment, increasing aeration and hence possibly activity of PAH attenuating bacteria. Vervaeke also reports higher degradation of mineral oil in the planted, as opposed to the fallow, site. Degradation of PAH is slower in reduced conditions than in aerobic conditions (McNally *et al.*, 1999). Degradation of PAH in an *in situ* anaerobic sediment will therefore be very slow and dredging to an aerobic environment would be needed for effective remediation (Quantin *et al.*, 2005).

7.1.2 The Canal Site

Site preparation has been described previously (Chapter 1, Royle *et al.*, 2003). In summary, naturally occurring vegetation was cleared from a 150 m stretch of the south bank of the Woolston New Cut Canal (WNCC) and a planting platform was created with sediment dredged from the canal bottom. This sediment was planted with willow (*Salix*), poplar (*Populus*) and alder (*Alnus*) species, as listed in Royle *et al.*, (2003), at a planting density of 27 000 trees per hectare. The design of the experiment consisted of 6 replicate blocks, each containing 13 treatment plots (tree species or no trees). These 13 treatments were randomly ordered within each block. Each plot measured 1.5 x 3 m and each "species" containing plot contained 12 trees, in two rows of 6 trees each, which were planted perpendicular to the canal (Appendix 6). One plot per block was not planted with trees and was used as a "control" plot. For the remainder of this chapter, for ease of reading, the plots where no trees are planted are encompassed in the word "species" when used to describe the layout of the canal site and the plots therein. For the plan of the species layout at the WNCC site, see Appendix 6. All plots were fully randomised within each block. Owing to the long and thin nature of the site, the blocks have had to be arranged in the appendix as individual blocks, shown three to a page. At the canal site the blocks ran in a line from east to west with Block A closest to the bridge (in the east) and Block F being the closest to the lock at the west end of the site. All plots in all blocks ran north/south from the canal wall to the canal water.

7.1.3 Aim

- To evaluate the viability of applying the phytoremediation technique trialled in the current project to the entire WNCC site, in order to create a clean soil from a contaminated sediment.

7.1.4 Hypothesis

The trees would establish at the canal site with a low mortality rate and grow evenly along the length of the canal. Due to the sediment being dredged onto the bank, no hotspots would be expected. However, if they did occur they would be identifiable by an area of high mortality or obvious toxicity symptoms. Phytoextraction and biomass production would be good, with the biomass *Salix* clones Tora and Calodendron producing the highest biomass. Due to the low economic value of the site, phytoextraction using SRC would prove to be a viable (but slow) option and could be applied to the length of the canal site.

7.2 Methods

7.2.1 Sediment Sampling

Sediment was sampled from along the length of the field site at the end of each growing season (Table 7.1). A random sampling design was rejected in favour of a modified herringbone design (DoE, 1994). The herringbone sampling pattern was applied to the trees so that a tree, and the sediment in which it was growing, could be sampled together, thus providing more meaningful results. Sediment cores were taken at approximately 10 cm from the tree base, so as to avoid root damage. At each sampling location, four 5 cm deep cores were taken using an auger. Two of the cores were sampled into polyethylene bags for metal analysis and other physico-chemical measures, and the other two were sampled into amber glass jars and stored at approximately 4 °C prior to analysis for organic contaminants.

7.2.2 Tree Sampling

Salix and *Populus* trees were coppiced at the end of the second growing season. All trees were then harvested at the end of the third growing season (Table 7.1 note that the decreasing number of samples taken was due to increased tree mortality). During these three growing seasons, the planting platform was weeded of naturally colonising vegetation. After the first growing season

Table 7.1 Dates of sampling of the sediment and trees on the WNCC planting platform.

Sampled	Date	Months post dredging	No. of Samples
Sediment	17/12/2002	8	166
	16/12/2003	20	166
	09/11/2004	31	166
Leaves	26/10/2002	6	104
	22/10/2003	18	94
	24/09/2004	29	71
Stems	26/01/2004	21	80
	24/09/2004	29	76

some dead or missing trees were replanted to maintain the planting density. Replanted trees were not sampled or analysed for contaminant content. Plots which were originally planted with the species *Salix atrocinerea*, which naturally colonises the WNCC, experienced 70 % mortality in the first growing season and the decision was made to abandon the "tree" treatments in these plots and allow them to naturally colonise with locally occurring vegetation. Thus weeding of these plots was not undertaken during the 2003 or 2004 growing seasons. The high mortality amongst the *Salix atrocinerea* suggests that this clone either grows poorly from cuttings or has no innate tolerance to heavy metals and thus may only colonise the site once the sediment has been exposed for a certain period of time.

The trees sampled alternated between tree numbers 4 and 7 and tree number 5 and 8 in consecutive plots and are highlighted in Appendix 6. Thus, one tree from near the wall and one tree from near the water were sampled from each plot.

Leaf samples were taken on the dates shown in Table 7.1. Leaves were sampled from the top 1/3 of the tree, or shoulder height, at all points around the compass where sufficient leaves remained (King, 2005). Due to the possibility of changing metal levels in tree leaves over time, it was important to standardise the time of year at which the leaves were taken (Vandecasteele *et al.*, 2002). This was done as far as possible. Tree mortality increased over time, leading to a fall in the number of leaf samples which could be collected. Stem samples were taken when the trees were coppiced. Fresh biomass was recorded in the field for willow and poplar species at the end of the 2003 and 2004 growing seasons, and for alders at the end of the 2004 growing season only. Branches of different sizes, representing the relative abundance of branch sizes on the tree, were taken for determination of dry weight and analysis for heavy metal content. Stem and leaf samples were dried at 60 °C until constant weight. Observations of apparent tree health – tree disease, herbivory, toxicity symptoms and relative growth were taken, and tree height and mortality were recorded on 24th September 2002 and 2004.

7.2.3 Sample Preparation

Sediment pH and conductivity were determined on subsamples of wet sediment in a 1:5 sediment-to-distilled water suspension, after 1 h of shaking and 0.5 h standing. A further subsample weighing between 3 and 5 g was dried at 105 °C until constant weight for determination of sediment moisture content. Loss on ignition was carried out on oven-dry sediment by heating the sediment to 480 °C for 8 h. The remaining wet sediment from the polyethylene bags was air-dried, ground, sieved to < 2 mm and stored in polyethylene bags.

7.2.4 Sequential Extraction

A 2-step sequential extraction procedure was applied to the sediment as a surrogate measure of "bioavailability" (Quevauviller *et al.*, 1996 a, Quevauviller *et al.*, 1996 b, Maiz *et al.*, 1997, French, 2005). For the extraction, 3 g of the air-dry sediment was weighed into 50 mL centrifuge tubes and 30 mL of 0.01 M $CaCl_2$ was added. Samples were shaken on an end-over-end shaker for 2 h, then centrifuged for 15 min at 3000 *g*. Supernatant was filtered through Whatman no. 542 filter papers into 15 mL polyethylene tubes. For the second step, 30 mL of 0.05 M EDTA was added to each sediment plug and samples were shaken on and end-over-end shaker for 1 h and then centrifuged at 3000 *g* for 15 min. Supernatant was filtered through Whatman no. 542 filter papers into 15 mL polyethylene tubes. Metal concentrations in the extracts were determined by ICP-AES.

7.2.5 Total Metal Analysis

7.2.5.1 Sediment

Approximately 0.5 g air-dry sediment was weighed into Teflon microwave vessels. Nine mL concentrated HNO_3 and 3 mL concentrated HCl were added to each sample. Samples were subjected to microwave-accelerated digestion in a MARS 5 microwave digester (CEM corp.). Samples were heated to 180 °C over the course of 10 min and held at this temperature for a further

10 min. After cooling, samples were filtered through Whatman no. 542 filter papers into 30 mL polyethylene tubes and made up to 25 mL with distilled water.

7.2.5.2 Leaf and Stem Samples

Dried stem samples were cut into small pieces by hand and further reduced in a blender. Leaves were crushed in the bags. Where available, 0.5 g of plant material was weighed into Teflon microwave vessels. Where 0.5 g was not available, the entire sample was used. Eight mL of concentrated HNO_3 and 2 mL of H_2O_2 were added to each sample. Samples were left to pre-digest for 5 min, or until reaction had ceased. Samples were digested and treated as described above.

7.2.5.3 Total Uptake of Elements

Total uptake of heavy metals into the biomass of the trees was calculated as follows. The canal site was divided in half lengthwise (i.e. into "top" and "bottom") (see Appendix 6) for measures of biomass (for reasons which will be explained in Chapter 8). One tree had been sampled from the "top" of each plot of trees and one from the "bottom" as previously described. The heavy metal contents of these sampled trees were multiplied by the dry weight biomass which had been collected for that half of the plot. For example, tree A 1.4 and tree A 1.7 were collected from the bottom and the top respectively of plot 1 in block A. The metal concentrations in the stems were applied to the dry weight of biomass which had been collected from plot A 1 bottom and A 1 top respectively. However, some of the trees which had been marked to be sampled had died and no stem was available for sampling although surrounding trees had survived and hence a biomass figure was present for that half of the plot. When this was the case, the metal concentrations used in the calculation were the mean values derived for that species/hybrid. Hence these data are presented as "estimated extra uptake" in Figures 7.3 to 7.5, due to the lack of actual metal concentrations.

Example: top half of plot: biomass = 3.5 t ha^{-1}, Zn concentration in stem = 250 mg kg^{-1}

= 875 g Zn ha^{-1} this value is counted as the true uptake of the clone.

Bottom half of the plot: biomass = 3.5 t ha^{-1}, Zn concentration in stem = missing. Therefore the mean value for that clone is used, which is, for example, 310 mg kg^{-1}. The uptake of Zn for this half of the plot is thus calculated as 1085 g Zn ha^{-1}, though as it is a best estimate it would appear on the graph as a component of the "estimated extra uptake" section.

7.2.5.4 Quality Control

To ensure accuracy of experimental results throughout the current project, one 0.5 g sample of a certified reference material (stream sediment GBW07312) was extracted, as described above, for every 24 samples extracted. These certified reference material samples were distributed throughout each ICP run. In the case of Cd, recovery was significantly higher than "total" and thus the Cd data were all corrected downwards accordingly. The proportion by which the data were reduced varied over the course of the experiment and the data from each ICP run were corrected according to the reference material recovery of Cd for that run. All other metals of interest here gave comparable recoveries over time, and none were above the certified "total" value. Under-recovery was difficult to judge as the certified "total" metal values were derived from a HF digest and not a HNO_3/HCl digest.

7.2.5.5 Organics Analysis

Sediment designated for organics analysis was sampled from the canal into amber glass jars. Samples were stored at < 4 °C until they were sent to an external, UKAS accredited laboratory (ALcontrol) for analysis. For determination of TPH the external laboratory undertook accelerated solvent extraction (hexane) with a Florisil® clean-up step. Analysis was carried out by GC-FID and the calibration standard was an alkane mixture. For determination of PAH a hexane extraction was carried out and clean-up was via the use of a suitable solid phase extraction cartridge. Analysis was by GC-FID. A thoroughly homogenised sediment standard was created in-house and sent to the laboratory as a sample to be analysed. It was included after every 20 true samples. These samples were used as a quality check and the laboratory also carried out internal quality checking procedures.

7.2.6 Statistical Analysis

The 2002 data were tested for any "block effects" due to lack of uniformity across the length of the canal site. At the end of the 2002 growing season there were no consistent, significant differences between the blocks and hence block data has been pooled to form replicates for each species/hybrid. Due this initial uniformity, data for 2003 and 2004 have been pooled in a like way. Any differences therein are due to the natural variation which the block structure was designed to account for and encompass. Results from all experimental blocks were therefore grouped according to species and checked for normality of distribution. None of the results presented in the current chapter followed normal distributions and hence non-parametric tests (Mann-Whitney U tests for 2 samples and Kruskall-Wallis analysis when > 2 samples) have been used throughout. Quoted p-values have been derived from these tests.

7.3 Results and Discussion

7.3.1 pH, Moisture Content and Conductivity

Sampling of the WNCC sediment from the planting platform was carried out in the winter months (November and December) when the sediment was at its wettest. This led to a mean moisture content in the WNCC sediment samples which was not significantly different between years ($p > 0.05$) (Table 7.2). The conductivity of the sediment however, decreased significantly between 2003 and 2004 (not measured in 2002), suggesting loss of soluble species and ions through leaching processes. The pH of the sediment was already low (pH 3.8) by the end of the first (2002) growing season, suggesting that the fall in pH during oxidation (as described in Chapters 3, 4 and 5) happened within the first 8 months of dredging. The pH did not drop further by 2004, suggesting that all of the sulphides exposed to the air had oxidised. When cores were taken from the canal site it was obvious that oxygen penetration was not ubiquitous. Only the top 0.5 cm or so of the sediment showed signs of oxidation. Below this level, the sediment was still black in colour, suggesting that it was reduced. Therefore, when oxidation of the deeper layers does occur, the pH would be expected to fall further. Equally, the pH, as determined during the wet winter months, may

Table 7.2 Mean pH, conductivity and moisture content of the WNCC sediment from 2002 to 2004. The pH of the sediment in 2004 was significantly different from the general mean in two cases and the identity and mean pH of these samples are shown in the footnote. For all other measures n = 166.

	End of growing season		
Mean Measure	**2002**	**2003**	**2004**
pH	3.81	3.7	3.92*
Conductivity (mS)	-	0.43	0.17
Moisture content (%)	104	101	105

* *Salix* "Calodendron" pH 4.18
No Trees planted pH 3.59

not be truly representative of the pH of the canal throughout the course of the year, due to the effect of redox conditions on pH. Unfortunately, the pH and moisture content of the WNCC sediment during the summer months were not established.

7.3.2 Sediment Heavy Metals

7.3.2.1 “Bioavailable” Heavy Metals

In the WNCC, the addition of the $CaCl_2$ and EDTA-extractable fractions did not correlate well with plant uptake of heavy metals in the majority of cases. Where correlation was present (e.g. *Populus* Ghoy stem Cd 2003 vs. “bioavailable” Cd, $r = 0.87$), it was not consistent between metals or years (e.g. *Populus* Ghoy stem Cd 2004 vs. “bioavailable” Cd, $r = - 0.50$). Thus extractants are examined separately, or in addition, but will not be considered to represent bioavailable metal. Metals extracted by $CaCl_2$ or EDTA separately, failed to correlate consistently with uptake of heavy metals into leaves or stems of the experimental tree species. Therefore, neither of these extractants alone accurately predicted bioavailability of heavy metals in this canal sediment. It should be remembered that the fraction extracted by EDTA will not be equal to that which would have been extracted by EDTA alone, as the sediment had first been extracted with $CaCl_2$.

7.3.2.2 Cadmium

Cadmium extractable in 0.01 M $CaCl_2$ remained at around 1 mg kg^{-1} over the three growing seasons (Fig. 7.1). However, 0.05 M EDTA-extractable Cd decreased significantly between 2002 and 2003 ($p < 0.001$), and 2003 and 2004 ($p < 0.001$). Total Cd in the soil also decreased significantly over this time period ($p < 0.001$). The decrease of total Cd between 2002 and 2004 was 5.9 mg kg^{-1}. The canal has a length of 2 km and is thought to contain approximately 40 000 t of sediment in need of dredging (Royle *et al.* 2003). Therefore, should this sediment all be removed from the canal bed as proposed, and be treated in the same manner as the sediment on the planting platform, the loss of total Cd over a 3 year period could be expected to be 236 kg. Cadmium as extracted in the 2-step sequential extraction forms a significant fraction of total Cd. In

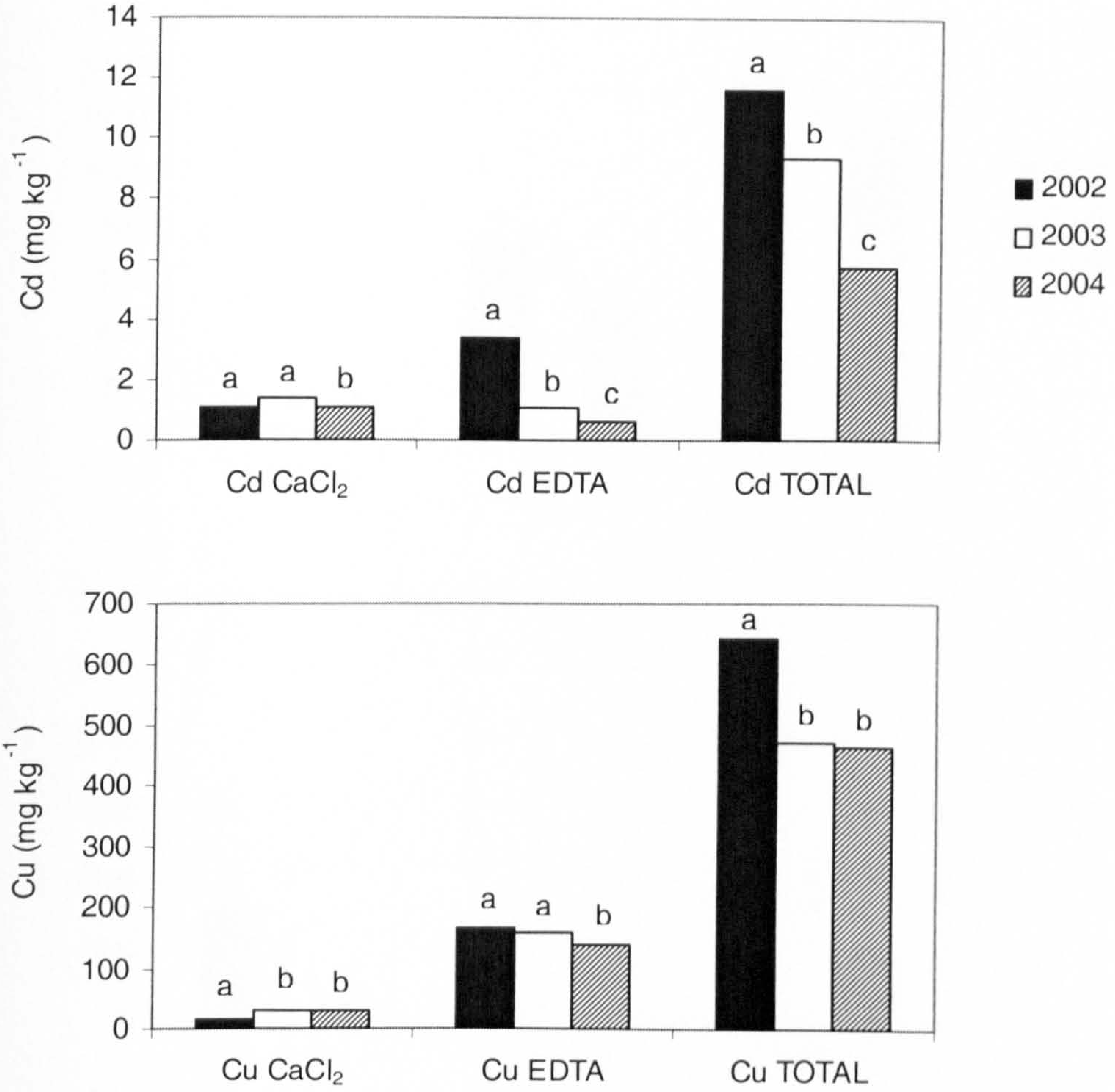


Figure 7.1 Cadmium and copper extracted from the WNCC sediment by $CaCl_2$, EDTA and HNO_3/HCl ("Total"), in 2002, 2003 and 2004. Values are mean (n = 166). Letters represent significant differences between years, for each extractant.

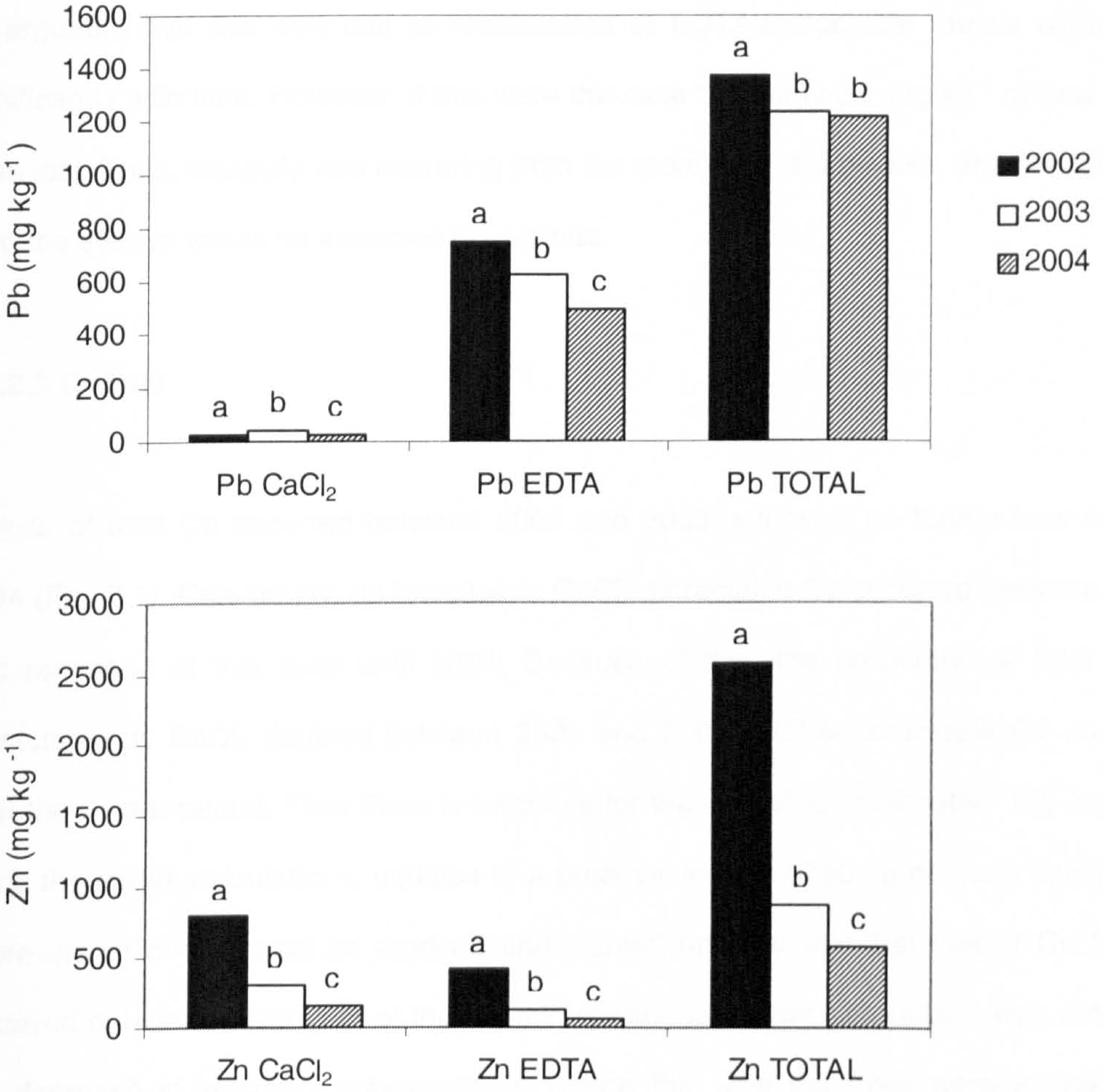


Figure 7.2 Lead and zinc extracted from the WNCC sediment by $CaCl_2$, EDTA and HNO_3/HCl digest ("Total"), in 2002, 2003 and 2004. Values are mean (n = 166). Letters represent significant differences between years, for each extractant.

2002 it accounted for 38 % of total Cd. In 2003 this figure was 26 % and by 2004 it had risen again to 30 %. This was a stable proportion when the loss of more that 50 % of total Cd during this time period is taken into account. It is suggestive of an equilibrium between the $CaCl_2$/EDTA-extractable pools of metals and the more strongly bound pools of metals. The consistency of the $CaCl_2$-extractable fraction suggests the maintenance of solution concentrations of Cd. It would perhaps be arguable that this was due to mobilisation of EDTA-extractable metals alone, as these fall significantly with time. However, if this were the case then only 3.1 mg kg^{-1} of total Cd would have been lost. Thus, resupply was occurring from the more resistant phases, and hence loss of total Cd from the system would be expected to continue.

7.3.2.3 Copper

A loss of total Cu occurred between 2002 and 2003, although no further loss had occurred by 2004 (Fig. 7.1). Conversely, an increase in $CaCl_2$-extractable Cu occurred between 2002 and 2003 and remained at this level until 2004. Because of this, the proportion of total Cu which was extractable by $CaCl_2$ doubled between 2002 and 2003. EDTA-extractable Cu decreased slightly over the 3-year period. Thus there is evidence for the loss of approximately 169 mg kg^{-1} Cu which, using the above calculations, equates to a potential loss of 6760 kg of Cu in one year, should the entire length of the canal be dredged and planted up. The fact that loss of Cu from the system occurred only in the first year of the experiment suggests that tree uptake was not responsible for the decrease in sediment concentration. During this time the trees were at their smallest, and hence they would be expected to have higher Cu uptake in 2003 or 2004 when growth would be expected to be more significant.

7.3.2.4 Lead

Total Pb in the WNCC sediment was high, but $CaCl_2$-extractable Pb reached a maximum of only 3.3 % of this (Fig. 7.2). EDTA-extractable Pb was much higher, at 55 % of total in 2002, falling to 41 % of total by 2004. As total Pb did not decrease significantly between 2003 and 2004, it is likely that the decrease in EDTA-extractable Pb was due to Pb immobilisation. However, such high concentrations of total and EDTA-extractable Pb may represent a toxic hazard, not just to the trees but also to soil-dwelling biota.

The decrease in total Pb concentrations from December 2002 to December 2003, though small, is significant and would equate to a loss of 5280 kg of Pb during the first year, if the entire canal were to be dredged and set up as in the current project.

7.3.2.5 Zinc

Total Zinc decreased exponentially between 2002 and 2003 and decreased again from 2003 to 2004 (Fig. 7.2). This was associated with a drop in both $CaCl_2$ and EDTA-extractable metals, although the proportion of the total which these constitute remains fairly stable at ~ 30 % and ~ 15 % respectively. The sediment on the WNCC planting platform lost 2006 mg of Zn per kg of sediment. If all 40 000 t of sediment were dredged and treated in this way, the loss of total Zn over 2 years would be expected to be 80.24 t.

7.3.2.6 General Sediment Metal Summary

Lead and Cu showed some signs of stabilisation after an initial loss of a proportion of total metal. As there were no significant differences between treatments on the canal site, it is unlikely that this stabilisation was due to phytostabilisation, as planted plots were not significantly different to unplanted plots. Cadmium and Zn showed the largest percentage losses due to their solubility and poor retention on sediment exchange sites. It is most probable that oxidation of the sediment led to the production of H_2SO_4 – hence the fall in pH from the original pH of 6.2 – releasing metals into more mobile forms as discussed in previous chapters. During wetter weather, the more soluble metals may be leached out of the system, thus decreasing total metals and also the more available forms. Uptake by the vegetation on the canal is also a likely source of loss of metals from the sediment.

Table 7.3 Tree biomass (dry weight) produced in the 2002-2003 growing seasons and the 2004 growing season, and total biomass production. Values are mean with standard deviations (SD). Stem density was 27 000 trees ha^{-1}.

	Biomass t ha^{-1} DW					
Species	**2002-03**	**SD**	**2004**	**SD**	**Total 2002-04**	**SD**
Alnus cordata					5.37	(8.39)
Alnus glutinosa					7.86	(8.88)
Alnus incana					3.95	(5.37)
Populus Ghoy	2.26	(2.09)	1.47	(1.91)	3.73	(1.95)
Populus Trichobel	4.40	(3.20)	3.20	(3.15)	7.60	(3.15)
Salix Ashton Stott	6.66	(9.06)	6.62	(10.1)	13.28	(9.41)
Salix Calodendron	8.70	(5.77)	9.19	(10.3)	17.89	(8.35)
Salix fragilis	3.95	(3.37)	3.51	(4.93)	7.46	(4.17)
Salix Jorrun	6.04	(5.28)	6.53	(8.21)	12.57	(6.79)
Salix Sericans	5.86	(4.00)	3.91	(3.64)	9.77	(3.86)
Salix Tora	6.44	(4.84)	8.30	(7.10)	14.74	(6.08)

7.3.3 Uptake of Metals by the Experimental Trees

7.3.3.1 Biomass

The poplar species produced higher biomass yields after the 2002/2003 growing seasons than the 2004 growing season (Table 7.3). No trees were coppiced at the end of the 2002 growing season due to their late planting. However, with the exception of *Salix fragilis* and *Salix* Sericans, willow clones tended to produce higher mean biomass during the 2004 growing season (Table 7.3). *Salix* Calodendron yielded the highest mean biomass of all of the experimental trees, though even this clone produced only 9.19 t ha^{-1} in the 2004 growing season (stool density 27 000 ha^{-1}). This biomass production is below that reported by other authors. Perttu and Kowalik (1997) quote normal biomass production of 10 – 12 t ha^{-1} y^{-1} DW though the stool density is not quantified. Mirck *et al.* (2005) report a stool density of around 20 000 per ha and a biomass production of > 10 t ha^{-1}. Vandenhove *et al.* (2001) investigated *Salix viminalis* ("Orm") growth on land contaminated with radionuclides on both sandy and loamy soil. Biomass production for season 1 was 10 and 8.5 t ha^{-1} DW respectively. In season 2 these figures increased to 12.9 and 12.1 t ha^{-1}. However, planting density was equivalent to 52 500 stools ha^{-1} in the first growing season and 42 500 stools ha^{-1} in the second. Laureysens *et al.* (2005), established a poplar SRC stand on a former household waste site. Biomass production was between 7.8 and 18.1 t ha^{-1} in 2 years, depending on the clone. *Populus* Trichobel yielded 12.9 ± 3.8 t ha^{-1}. On the WNCC site, *Populus* Trichobel yielded only 7.6 ± 3.2 t ha^{-1} over 3 years. Biomass production on the WNCC site was lower than suggested for biomass fuel production (Perttu and Kowalik, 1997), but within the range which has been found by other authors. Mean biomass production of *Populus* Ghoy and *Alnus incana* was low (< 4 t ha^{-1}) though the standard deviation of the *A. incana* data was high, meaning that *Populus* Ghoy exhibited the poorest growth of all of the species selected (except for the failed *S. atrocinerea*). Most experimental trees exhibited a high degree of variability in biomass production, and this phenomenon will be discussed in more detail in Chapter 8.

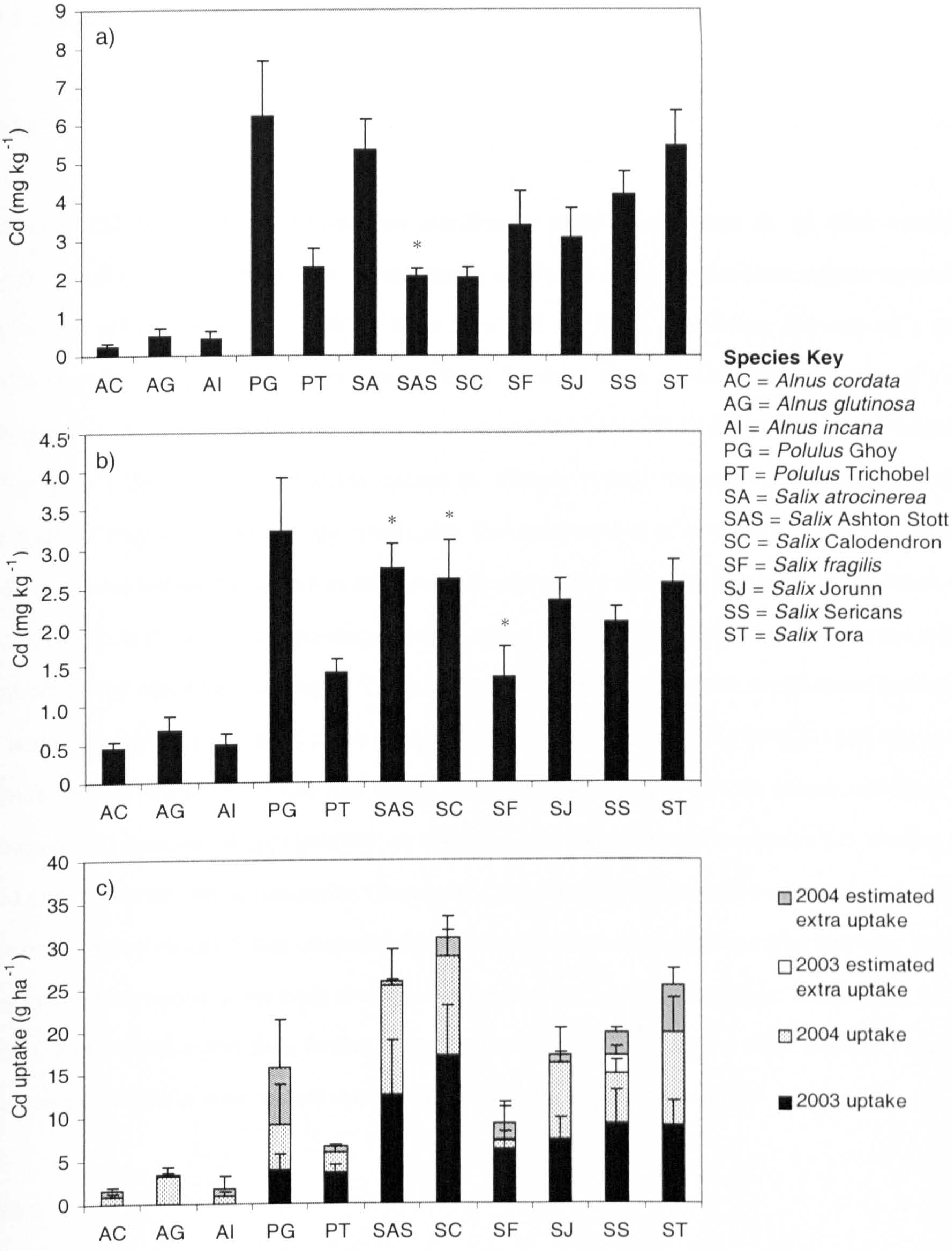


Figure 7.3 Cadmium concentrations in a) experimental tree leaves b) experimental tree wood and c) total uptake of metal in tree biomass per hectare of planted canal. Values are mean + standard errors, n varies (see Table 7.1). "Estimated extra uptake" in c) has been calculated from the mean of uptake for that year and the "remaining" biomass (see text). *Alnus* species were harvested in 2004 only and values for these species represent total uptake over the 3 growing seasons. A " * " marks species where metal accumulation in leaves/wood was significantly different between years. Values for these species can be found in Appendix 8.

7.3.3.2 Cadmium

Leaves

Uptake of Cd by the *Alnus* species was significantly lower than uptake by all other species ($p < 0.001$) (Fig 7.3 a). There was no significant difference in mean Cd foliar uptake between poplar and willow species ($p = 0.405$). *Salix* Tora (5.5 mg kg^{-1}), *atrocinerea* (5.4 mg kg^{-1}) and Sericans (4.2 mg kg^{-1}) took up significantly more Cd than *Salix* Ashton Stott (2.1 mg kg^{-1}) or Calodendron (2.1 mg kg^{-1}). Theses values are between the "normal" (< 2.4 mg kg^{-1}) and "critical" (> 5 mg kg^{-1}) Cd foliar concentrations quoted by Alloway (1995). Though Alloway's values are generalised, they can act as a guide in this case. Vandecasteele *et al.* (2005) monitored Cd uptake in *Salix cinerea* leaves throughout three growing seasons. The sediment had a total Cd content of 14.2 mg kg^{-1}. At the end of the growing seasons, mean Cd concentrations in the *Salix* leaves were generally in the range 20 to 27 mg kg^{-1}, with the exception of one tree which accumulated between 37 and 65 mg kg^{-1}. Compared to these values, uptake into the leaves of the WNCC trees was low. Uptake of high levels of Cd into leaf tissue is not desirable at the WNCC unless leaf litter is collected and disposed of appropriately, as breakdown of leaf litter may re-release Cd, leading to food chain transfer and accumulation (Vervaeke *et al.*, 2003 and references cited therein). High concentrations of metals in leaf material may increase the severity of any food chain transfer which may occur if the leaves of the trees are eaten by herbivorous animals or insects. Thus, of the trees tested, *Alnus* species and *Salix* Ashton Stott and Calodendron may be the most suitable trees to grow on the WNCC in order to limit dispersal of Cd into the wider environment.

Stems

Of all of the experimental trees, *Alnus* species had the lowest concentration of Cd in their woody tissues (Fig. 7.3 b). Values expressed in this figure represent metal concentrations in the stems of poplar and willow species at the end of each growing season. Alders were not harvested until after the 2004 growing season and hence heavy metal concentrations in *Alnus* stems are cumulative, whereas *Populus* and *Salix* metal concentrations are not. The lower Cd concentrations in alder stems may be because the trees were not coppiced at the end of the 2003 growing season and thus did not undergo the burst of growth experienced by the willow and poplar species. *Populus* Ghoy took up significantly more Cd (3.2 mg kg^{-1}) than *Populus* Trichobel (1.4 mg kg^{-1}) ($p < 0.05$). Contrary to the leaf concentrations of Cd, *Salix* Ashton Stott and Calodendron took up the highest mean concentrations of Cd into their woody biomass. There was no statistical difference between stem concentrations of Cd between willow clones.

These data suggest that *Populus* Trichobel, *Salix fragilis* and the *Alnus* species would be the least effective trees for phytoextraction of Cd in this canal sediment.

Total Uptake of Cd

Total estimated uptake of Cd by the experimental tree species on the WNCC site is given in Figure 7.3 c. Due to the different survivorship and biomass production between species and clones, those trees which had the highest concentration of Cd in their woody biomass (mg kg^{-1}) did not necessarily exhibit the highest total uptake (g ha^{-1}) of Cd. Uptake of Cd by *Alnus* species was very low when compared to uptake by *Populus* and *Salix* species. The three trees with the highest total uptake of Cd were *Salix* Calodendron (31 g ha^{-1}), *Salix* Ashton Stott (26 g ha^{-1}) and *Salix* Tora (25 g ha^{-1}). *Salix* Calodendron produced higher mean biomass than *Salix* Tora and *Salix* Ashton Stott (Table 7.3) and, though stem concentrations were not significantly different, foliar Cd concentrations were significantly higher in *Salix* Tora than *Salix* Calodendron. This would increase the potential of food chain transfer of Cd in *Salix* Tora with respect to Calodendron or Ashton Stott.

Populus Ghoy was the most effective poplar phytoextractor of Cd, and *Alnus glutinosa* performed best out of the alders. *Populus* Trichobel grown on the WNCC site over 3 growing seasons extracted a total of 6.6 g Cd ha^{-1}. Laureysens *et al.* (2005) found accumulation of 12 g Cd ha^{-1} in *Populus* Trichobel grown on a former household waste disposal site, after 2 growing seasons. Trichobel therefore performed poorly on the WNCC site, suggesting unfavourable soil conditions or element availability, for phytoextraction. *Salix* Calodendron extracted a mean total of approximately 32 g Cd ha^{-1} in 3 years, equivalent to 24.6 g Cd from the entire canal site, were it dredged and

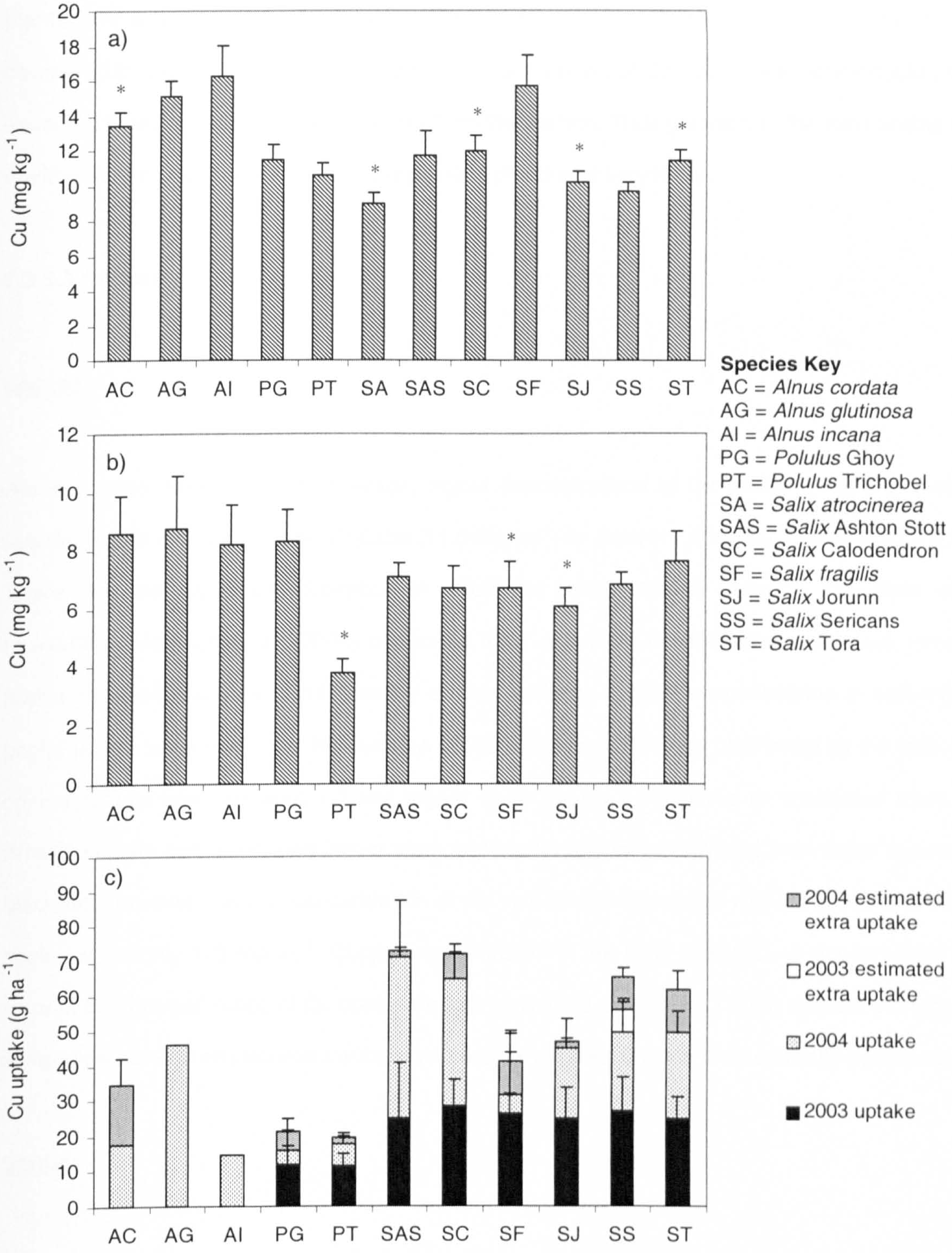


Figure 7.4 Copper concentrations in a) experimental tree leaves b) experimental tree wood and c) total uptake of metal in tree biomass per hectare of planted canal. Values are mean + standard errors, n varies (see Table 7.1). "Estimated extra uptake" in c) has been calculated from the mean of uptake for that year and the "remaining" biomass (see text). *Alnus* species were harvested in 2004 only and values for these species represent total uptake over the 3 growing seasons. A " * " marks species where metal accumulation in leaves/wood was significantly different between years. Values for these species can be found in Appendix 8.

planted as was done in the present project. Total loss of Cd from 40 000 t of sediment as calculated in section 7.3.3.2 would be 236 kg. Thus removal of Cd by phytoextraction could only be expected to account for 0.01 % of Cd lost from the system. Thus pollution of the surrounding canal environment by leaching of Cd from the planting platform is very likely.

7.3.3.3 Copper

Leaves

Alnus species' leaves had, on average, higher concentrations of Cu (14.9 mg kg^{-1}) in their leaf tissues (Table 7.4) than did the *Populus* (11.0 mg kg^{-1}) or *Salix* (11.4 mg kg^{-1}) species ($p < 0.001$). There was no significant difference in foliar Cu concentrations between the *Salix* clones ($p = 0.083$). Madejón *et al.* (2004) compared foliar and stem concentrations of heavy metals in poplar trees affected and unaffected by a mine spillage. Copper concentrations in spill-affected poplar leaves appeared not to be elevated above those found in trees unaffected by the spill. Stem concentrations however were 1.7 fold higher (6.62 mg kg^{-1}) compared to unaffected trees. The WNCC poplars both contained higher stem and higher leaf concentrations than those reported by Madejón. However, total Cu concentration of the spill-contaminated soil was lower than the WNCC sediment, at only 179 mg kg^{-1}. Copper concentration in the foliar tissue of all experimental trees were in the "normal" range of Cu concentrations as quoted by Alloway (1995) and are therefore not likely to be causing any serious toxic effects such as reduced yield or visible toxicity symptoms.

Stems

There were no significant interspecies differences between the stem Cu concentrations of *Alnus* and *Salix* species ($p > 0.05$). However, it should be remembered that *Alnus* stem concentrations are cumulative and *Salix* concentrations are not. *Populus* Trichobel had significantly lower stem Cu concentrations than did *Populus* Ghoy ($p < 0.01$) and showed the lowest stem Cu concentrations of any of the experimental trees. Copper concentrations in the stems of the experimental trees was within the "normal" range (Alloway, 1995).

Total Uptake of Cu

Populus species were the least viable for phytoextraction of Cu. The *Salix* clones showed the highest phytoextraction potential, accumulating average Cu concentrations of up to 80 g ha^{-1}. The entire WNCC south bank has an area of 0.77 ha, meaning that maximum mean uptake of Cu, should the length of the site be dredged, would be 62 g of Cu over the 3 year period. However, loss of Cu from the site over this same period would be 6760 kg (as calculated in section 7.3.3.3). Phytoextraction would therefore account for only a tiny fraction of the total Cu which would be lost. On the experimental site it is almost certain that the loss in total Cu from the site was not due to phytoextraction, but loss by other methods such as leaching. Thus pollution of the canal water and environment would be expected to occur.

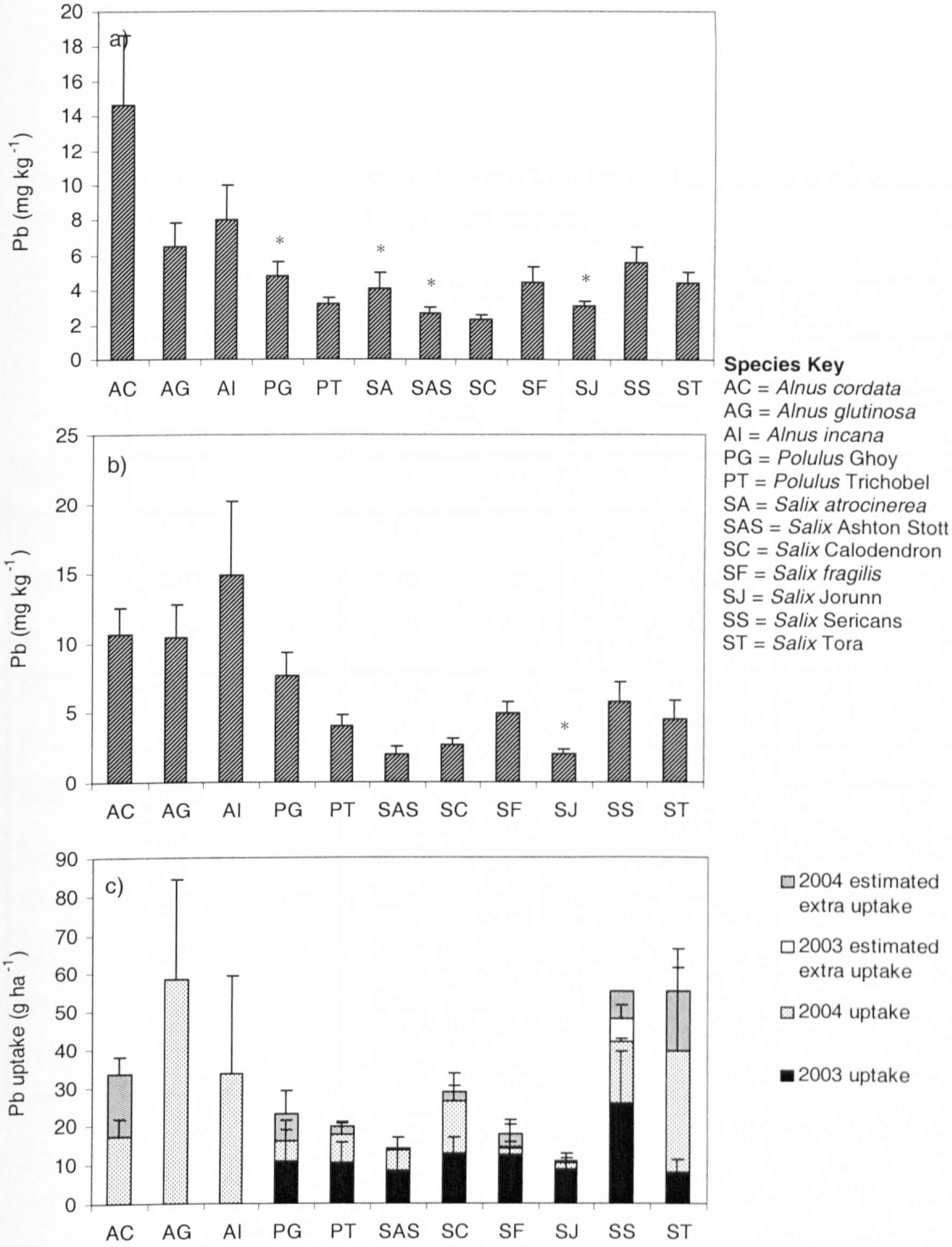
7.3.3.4 Lead

Leaves

Alnus cordata exhibited the highest mean foliar concentrations of Pb. However the standard deviation from the mean was very large (14.6 ± 22 mg kg^{-1}). *Populus* Ghoy accumulated higher concentrations of Pb in its foliar tissue than did *Populus* Trichobel (Fig. 7.5 a). *Salix* Sericans and *Salix* Tora had significantly higher Pb concentrations in their leaves than *Salix* Ashton Stott, *Salix* Calodendron and *Salix* Jorunn, which together had the lowest concentration of Pb in the leaves of all species and clones. The bioconcentration factor (BCF) of Pb in foliar tissue was < 0.01 in all species except *Alnus cordata* (BCF = 0.01) (Table 7.4). All foliar concentrations of Pb were within the "normal" range (0.2 – 20 mg kg^{-1}) (Alloway, 1995).

Stems

The *Alnus* species exhibited higher accumulation of Pb in their woody tissues than *Populus* or *Salix* species (Fig. 7.5 b). However, *Alnus* values are cumulative as the stems were only harvested in



Species Key

AC = *Alnus cordata*
AG = *Alnus glutinosa*
AI = *Alnus incana*
PG = *Polulus* Ghoy
PT = *Polulus* Trichobel
SA = *Salix atrocinerea*
SAS = *Salix* Ashton Stott
SC = *Salix* Calodendron
SF = *Salix fragilis*
SJ = *Salix* Jorunn
SS = *Salix* Sericans
ST = *Salix* Tora

Figure 7.5 Lead concentrations in a) experimental tree leaves b) experimental tree wood and c) total uptake of metal in tree biomass per hectare of planted canal. Values are mean + standard errors, n varies (see Table 7.1). "Estimated extra uptake" in c) has been calculated from the mean of uptake for that year and the "remaining" biomass (see text). *Alnus* species were harvested in 2004 only and values for these species represent total uptake over the 3 growing seasons. A " * " marks species where metal accumulation in leaves/wood was significantly different between years. Values for these species can be found in Appendix 8.

Table 7.4 The bioconcentration factors of Cd and Zn in the wood and leaves of the experimental trees at the end of the 2003 and 2004 growing seasons.

	Bioconcentration Factor							
	Wood				**Leaves**			
	2003		**2004**		**2003**		**2004**	
Species	**Cd**	**Zn**	**Cd**	**Zn**	**Cd**	**Zn**	**Cd**	**Zn**
AC	0.05	0.46	0.08	0.69	0.03	0.63	0.04	0.95
AG	0.07	0.37	0.12	0.56	0.05	0.82	0.09	1.23
AI	0.05	0.53	0.09	0.80	0.05	0.90	0.08	1.36
PG	0.35	0.44	0.57	0.67	0.67	1.86	1.10	2.80
PT	0.15	0.29	0.25	0.43	0.25	0.99	0.41	1.49
SAS	0.30	0.51	0.48	0.76	0.22	1.28	0.36	1.93
SC	0.28	0.43	0.46	0.65	0.22	1.15	0.36	1.73
SF	0.15	0.41	0.24	0.62	0.36	1.29	0.59	1.95
SJ	0.25	0.40	0.41	0.61	0.33	1.24	0.54	1.86
SS	0.22	0.51	0.36	0.77	0.45	2.12	0.74	3.19
ST	0.28	0.51	0.45	0.78	0.59	1.78	0.96	2.68

Species Key
AC = *Alnus cordata*
AG = *Alnus glutinosa*
AI = *Alnus incana*
PG = *Polulus* Ghoy
PT = *Polulus* Trichobel
SAS = *Salix* Ashton Stott
SC = *Salix* Calodendron
SF = *Salix fragilis*
SJ = *Salix* Jorunn
SS = *Salix* Sericans
ST = *Salix* Tora

2004. Bioconcentration of Pb in stems was also low with a BCF of ≤ 0.01 (data not shown). Lead concentrations in stems did not exceed the "normal" values quoted by Alloway (1995).

Total Uptake of Pb

The three tree species which exhibited the highest phytoextraction of Pb were *Alnus glutinosa* (59 g ha^{-1}, though the standard deviation of these data was large), *Salix* Sericans (55 g ha^{-1}) and *Salix* Tora (55 g ha^{-1}) (Fig. 7.5 c). The highest total uptake of Pb by the experimental trees accounted for a negligible portion of the total Pb which was lost from the sediment, again suggesting pollution of the surrounding canal environment.

7.3.3.5 Zinc

Leaves

Salix Sericans exhibited significantly higher accumulation of Zn in its foliar tissues than most other clones ($p < 0.001$), with the exception of *Salix* Tora (Fig. 7.6 a). Leaf concentrations of Zn were generally high. Alloway (1995) quotes the following values for mature leaf concentrations: Deficient = < 10 - 20 mg kg^{-1}; Sufficient = 25 - 150 mg kg^{-1}; Excessive or toxic = > 400 mg kg^{-1}. It is therefore likely that all of the species on the WNCC site were experiencing Zn toxicity and may have been showing toxicity symptoms and experiencing reduced yields (Alloway, 1995). Zinc is the only metal studied in the current chapter, where mean foliar metal contents are classed by Alloway as "critical" across all species. It is therefore logical to conclude that the toxicity symptoms visible in the experimental trees and quantified in Appendix 7 and Figures 7.7 and 7.8, were an effect of Zn toxicity. However, mean foliar concentrations of Cd also reached the lower end of the "critical" range in *Populus* Ghoy, *Salix* Ashton Stott and *Salix* Tora, and so in these 3 tree species Cd toxicity may also have been a factor.

Vandecasteele *et al.* (2005) report foliar concentrations of between 1000 and 2500 mg kg^{-1} Zn in the leaves of *Salix cinerea* grown on seasonally flooded, contaminated sediment. The highest

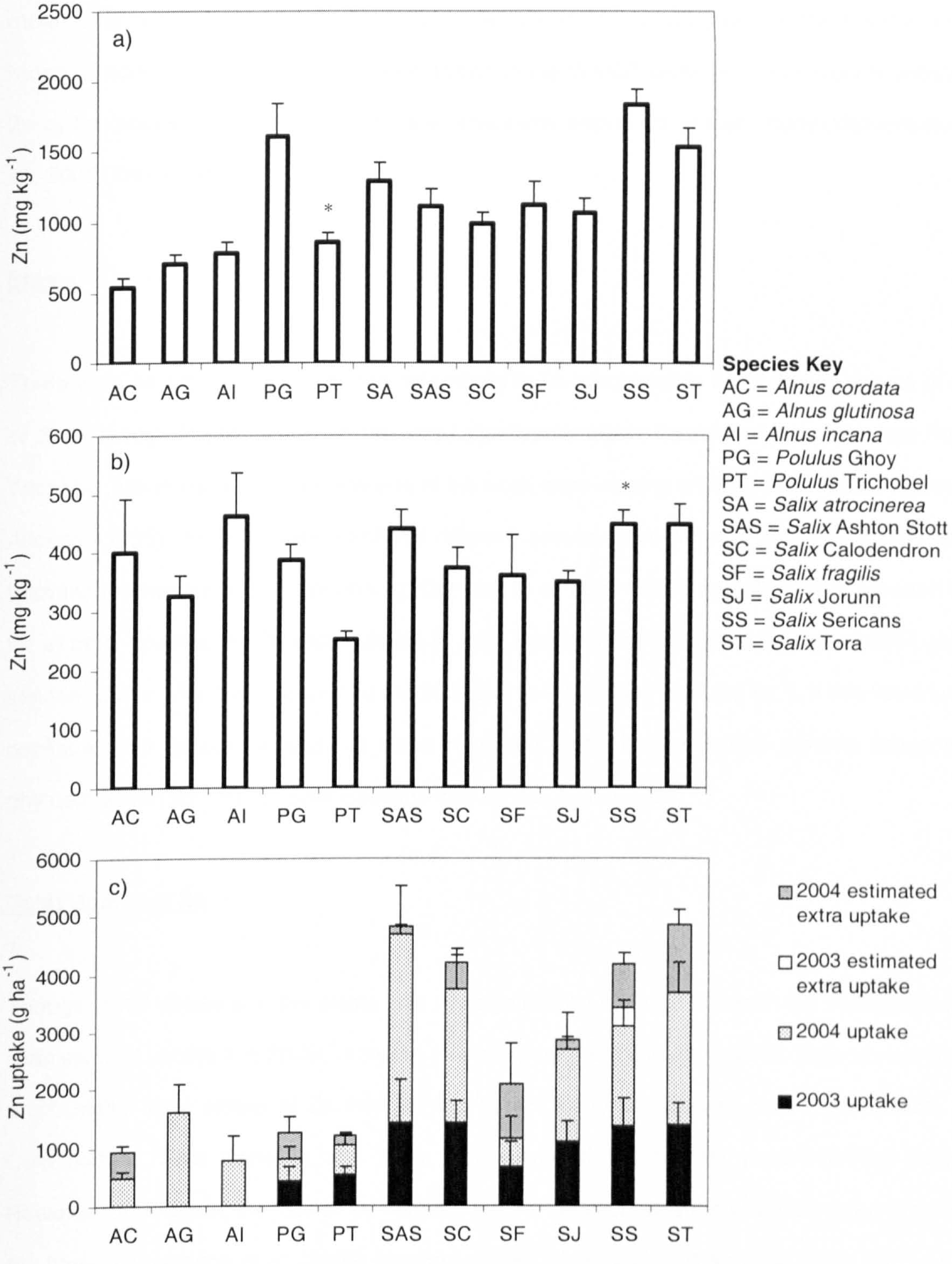


Figure 7.6 Zinc concentrations in a) experimental tree leaves b) experimental tree wood and c) total uptake of metal in tree biomass per hectare of planted canal. Values are mean + standard errors, n varies (see Table 7.1). "Estimated extra uptake" in c) has been calculated from the mean of uptake for that year and the "remaining" biomass (see text). *Alnus* species were harvested in 2004 only and values for these species represent total uptake over the 3 growing seasons. A " * " marks species where metal accumulation in leaves/wood was significantly different between years. Values for these species can be found in Appendix 8.

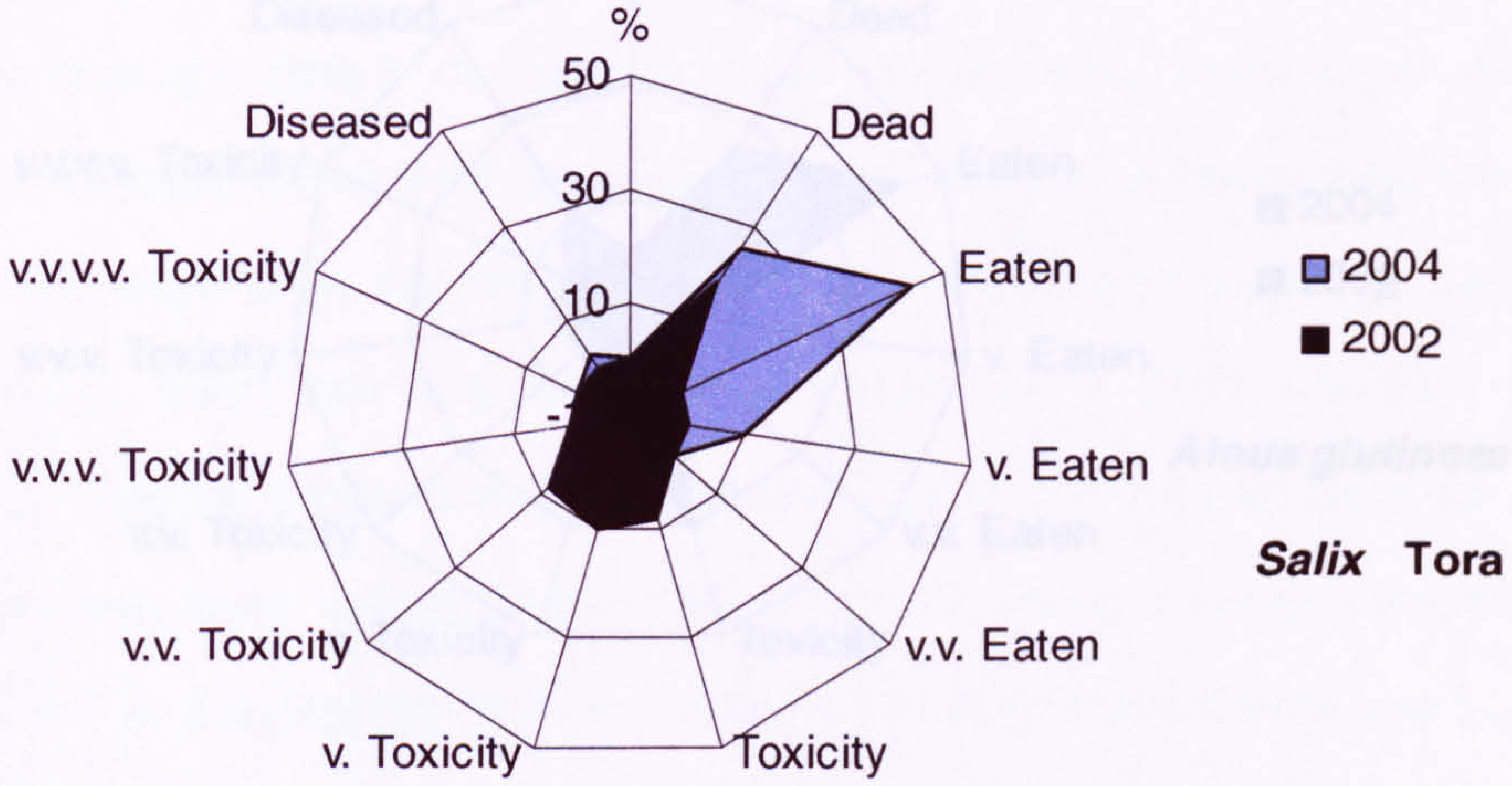
mean foliar concentrations of Zn in the experimental trees in this study were at the lower end of this range, despite the higher total Zn concentration in the WNCC sediment. Thus bioconcentration of Zn by the trees was higher in the Vandecasteele study than in the present study (Vandecasteele *et al.*, 2005, Table 7.4).

Stems

There were no significant interspecies differences in Zn accumulation in the woody tissues of *Alnus* or *Salix*, though *Populus* Ghoy accumulated significantly higher concentrations of Zn than *Populus* Trichobel. Mean stem Zn concentrations of the trees were verging on the critical values reported by Alloway (1995). *Salix* Sericans exhibited different accumulation rates of Zn in its woody tissues between the two harvests (Appendix 8). Contrary to all other significant differences between years for all other species, the Zn accumulation in *Salix* Sericans was increased during the 2004 growing season (531 mg kg^{-1}) with respect to the 2002/3 growing season (364 mg kg^{-1}). If this trend were to continue, or if uptake remained at these increased levels in consecutive growing seasons, the phytoextraction potential of *Salix* Sericans may be much increased.

Total Uptake of Zn

Though metal uptake into the stems was not significantly different between the experimental tree species, total uptake in g Zn ha^{-1} showed a much more pronounced variation. *Salix* clones showed much higher total uptake of Zn than either *Populus* or *Alnus* species. *Salix* Ashton Stott, *Salix* Calodendron, *Salix* Sericans and *Salix* Tora exhibited the highest phytoextraction potential. However, once again, the loss of Zn from the system far exceeded the phytoextraction potential of the trees. Laureysens *et al.* (2005) reported uptake of between 0.6 and 2.4 kg Zn ha^{-1} in poplar clones. They report accumulation in *Populus* Trichobel of 1.0 ± 0.3 kg Zn ha^{-1} over 2 growing seasons. Trichobel grown on the WNCC accumulated 1.2 kg Zn ha^{-1} in 3 growing seasons. Vervaeke *et al.* (2003) estimate that with an annual SALIMAT biomass production of 11.5 DM t ha^{-1}, extraction of Zn after 3 years would be 5 kg ha^{-1}. This is similar to the extraction of Zn from the WNCC sediment by *Salix* Ashton Stott and *Salix* Tora.



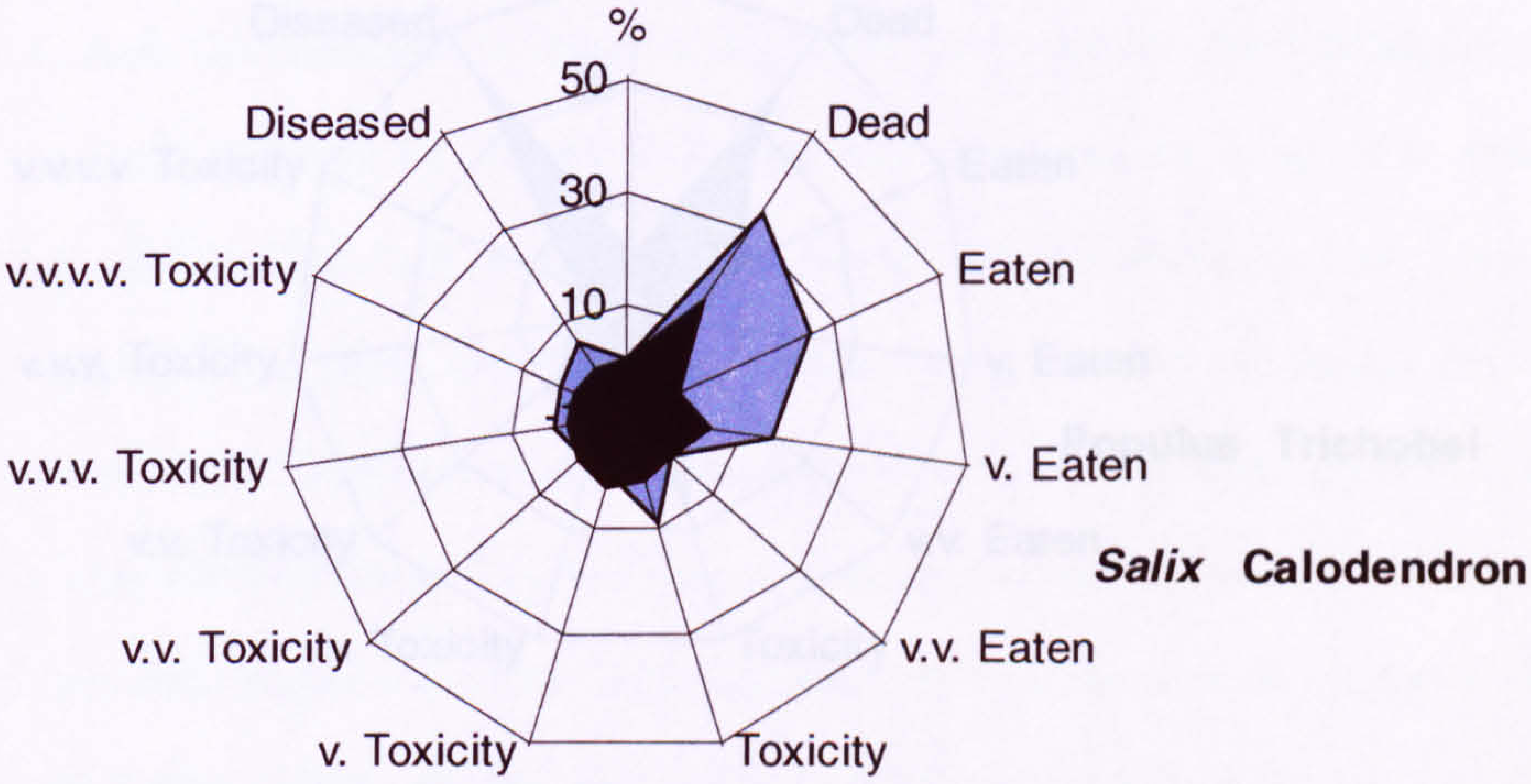


Figure 7.7 The relative changes in the percentage of *Salix* Tora and *Salix* Calodendron trees which exhibited the cited symptoms, between 2002 and 2004. For key, see Appendix 7.

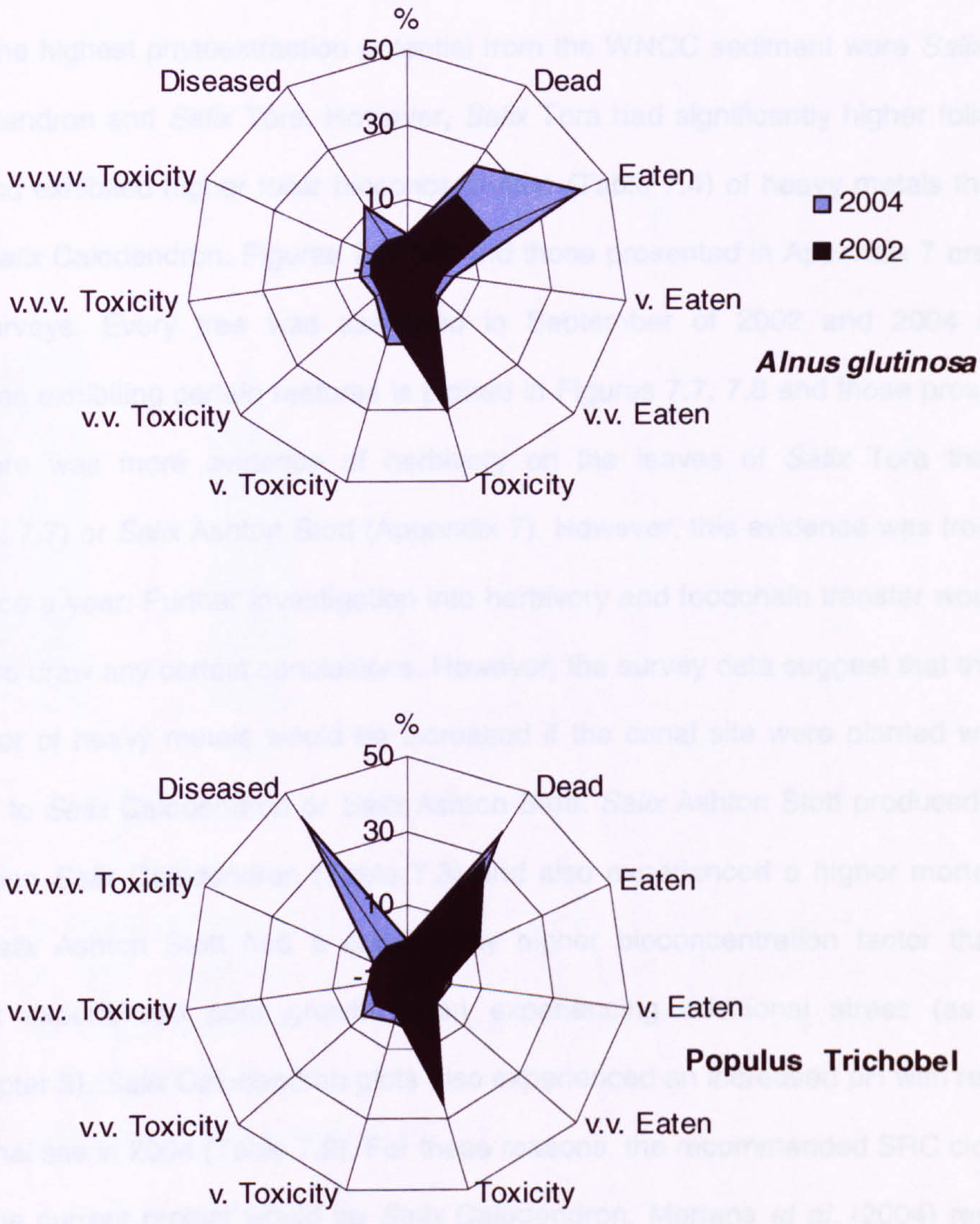


Figure 7.8 The relative changes in the percentage of *Alnus glutinosa* and *Polulus* Trichobel trees which exhibited the cited symptoms, between 2002 and 2004. For key, see Appendix 7.

7.3.4 Phytoextraction Potential

The clones with the highest phytoextraction potential from the WNCC sediment were *Salix* Ashton Stott, *Salix* Calodendron and *Salix* Tora. However, *Salix* Tora had significantly higher foliar metal concentrations and exhibited higher foliar bioconcentration (Table 7.4) of heavy metals than *Salix* Ashton Stott or *Salix* Calodendron. Figures 7.7, 7.8 and those presented in Appendix 7 are results of two visual surveys. Every tree was assessed in September of 2002 and 2004 and the percentage of trees exhibiting certain features is plotted in Figures 7.7, 7.8 and those presented in Appendix 7. There was more evidence of herbivory on the leaves of *Salix* Tora than *Salix* Calodendron (Fig. 7.7) or *Salix* Ashton Stott (Appendix 7). However, this evidence was from visual surveys taken once a year. Further investigation into herbivory and foodchain transfer would have to be carried out to draw any certain conclusions. However, the survey data suggest that the risk of food chain transfer of heavy metals would be increased if the canal site were planted with *Salix* Tora as opposed to *Salix* Calodendron or *Salix* Ashton Stott. *Salix* Ashton Stott produced a lower mean biomass than *Salix* Calodendron (Table 7.3) and also experienced a higher mortality rate (Appendix 7). *Salix* Ashton Stott had a consistently higher bioconcentration factor than *Salix* Calodendron but experienced poor growth when experiencing additional stress (as will be discussed in Chapter 8). *Salix* Calodendron plots also experienced an increased pH with respect to the rest of the canal site in 2004 (Table 7.2). For these reasons, the recommended SRC clone from those tested in the current project would be *Salix* Calodendron. Mertens *et al.* (2004) report that *Alnus glutinosa* planted on a saline dredged sediment showed repressed growth and accumulated levels of heavy metals which would be classed by Alloway (1995) as normal, suggesting that it would be better used for phytostabilisation than phytoextraction. In the current study, *Alnus glutinosa* showed much increased herbivory in 2004 compared to 2002, a higher mortality and the presence of disease (Fig. 7.8). These factors will decrease the expected viability of this tree as a phytoextractor at the WNCC site. Forty percent of the experimental *Populus* Trichobel trees were also blighted by disease in 2004. *Alnus* and *Populus* species exhibited toxicity symptoms in a greater proportion of experimental trees than the *Salix* clones (Appendix 7), though this decreased from 2002 to 2004, perhaps due to the decrease in sediment total metal concentrations.

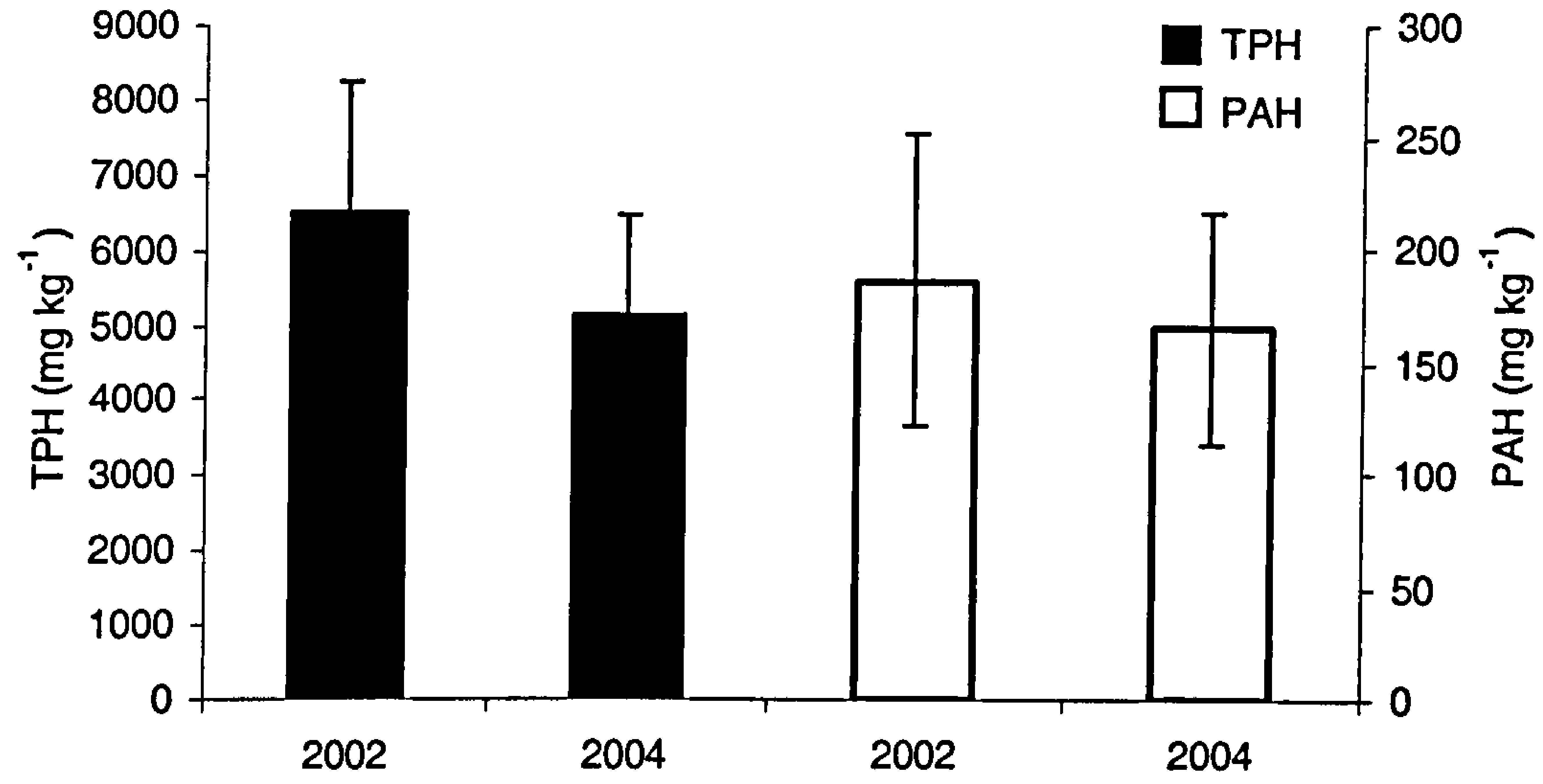


Figure 7.9 Organic contaminant concentrations on the WNCC site in 2002 and 2004. Values are mean ± SD (n = 83). TPH = Total Petroleum Hydrocarbons. PAH = Polycyclic Aromatic Hydrocarbons. The 2002 values represent concentrations at 8 months after dredging. The 2004 values represent concentrations at 21 months after dredging. For values at 2 months after dredging see the "Alcontrol" results in Appendix 1.

The loss of contaminated leachate from the planting platform may adversely affect the germination and growth of surrounding vegetation (Bedell *et al.*, 2003). However, stands of SRC trees can have very high transpiration rates thus reducing leachate (Vervaeke *et al.*, 2001). Licht and Isebrands (2005) report figures for water consumption by poplar trees of between 20 and 50 kg of water per tree per day, or 69 600 L ha^{-1} d^{-1}. A more viable scheme for phytoremediation of the WNCC may therefore be to add a surface layer of contaminated sediment to a stand of SRC trees in their second growing season. Thus leaching from the site could be reduced and metal uptake may be increased in the first year post-dredging with respect to planting willow pegs on newly-dredged sediment. Mortality and toxicity may also be reduced.

7.3.5 Phytoremediation of Organic Pollutants

The different species treatments planted on the WNCC site did not significantly effect the concentrations of TPH or PAH in the sediment in 2002 ($p = 0.677$ and 0.501 respectively) or in 2004 ($p = 0.261$ and 0.702 respectively). Greater losses of PAH from fallow as opposed to planted plots have been reported (Vervaeke *et al.*, 2003), though for this site that was not proved to be true. Between 2002 and 2004, TPH concentration on the canal site decreased significantly ($p < 0.001$) (Fig. 7.9). PAH concentration did not decrease significantly between 2002 and 2004 ($p = 0.067$), though the mean PAH concentration did decrease. The rate of disappearance of the organic pollutants was slow compared to other methods of degradation such as composting of the sediment (Semple, 2001), which may be a more viable option for treatment of this sediment. The presence of weathered oil may affect the behaviour of PAH in the sediment system. At concentrations of 100 to 1000 mg kg^{-1} competitive interactions have been reported (Jonker *et al.*, 2003) between oil and PAH for adsorption sites on the sediment organic matter. Above these concentrations, the presence of oil may enhance the adsorption of PAH, thus reducing PAH bioavailability and hence breakdown rate (Jonker *et al.*, 2003). It is therefore possible that the presence of the high concentrations of TPH in the WNCC sediment (~ 6500 mg kg^{-1}) may have caused a reduction in the bioavailability of PAH, leading to the smaller than expected mean reduction in PAH concentration in the sediment.

7.4 General Conclusions

The WNCC site underwent significant acidification within the first 8 months post-dredging. It is probable that this oxidation and acidification led to increased heavy metal availability and hence loss from the planting platform sediment. The winter pH of the sediment then remained fairly stable and the less mobile heavy metals (Cu and Pb) experienced no further significant loss in total concentration during the course of the current project. However, the more mobile heavy metals (Cd and Zn) did continue to leach from the planting platform into the surrounding environment and may potentially have polluted this environment.

The planted trees did not grow as well as expected and growth was very variable across the site. Due to the low uptake of heavy metals (compared to values quoted in the literature) phytoremediation of the site using phytoextraction is not a viable option. However, of the tree species examined, *Salix* Calodendron would be the most effective tree for phytoextraction of heavy metals from the WNCC sediment.

Phytodegradation of the organic pollutants did occur. However, due to the lack of efficiency of the heavy metal phytoremediation, composting may be a more viable option for the removal of organic pollutants. Collection of leachate and wastewater from the compost would be necessary to avoid pollution of the surrounding environment.

CHAPTER 8

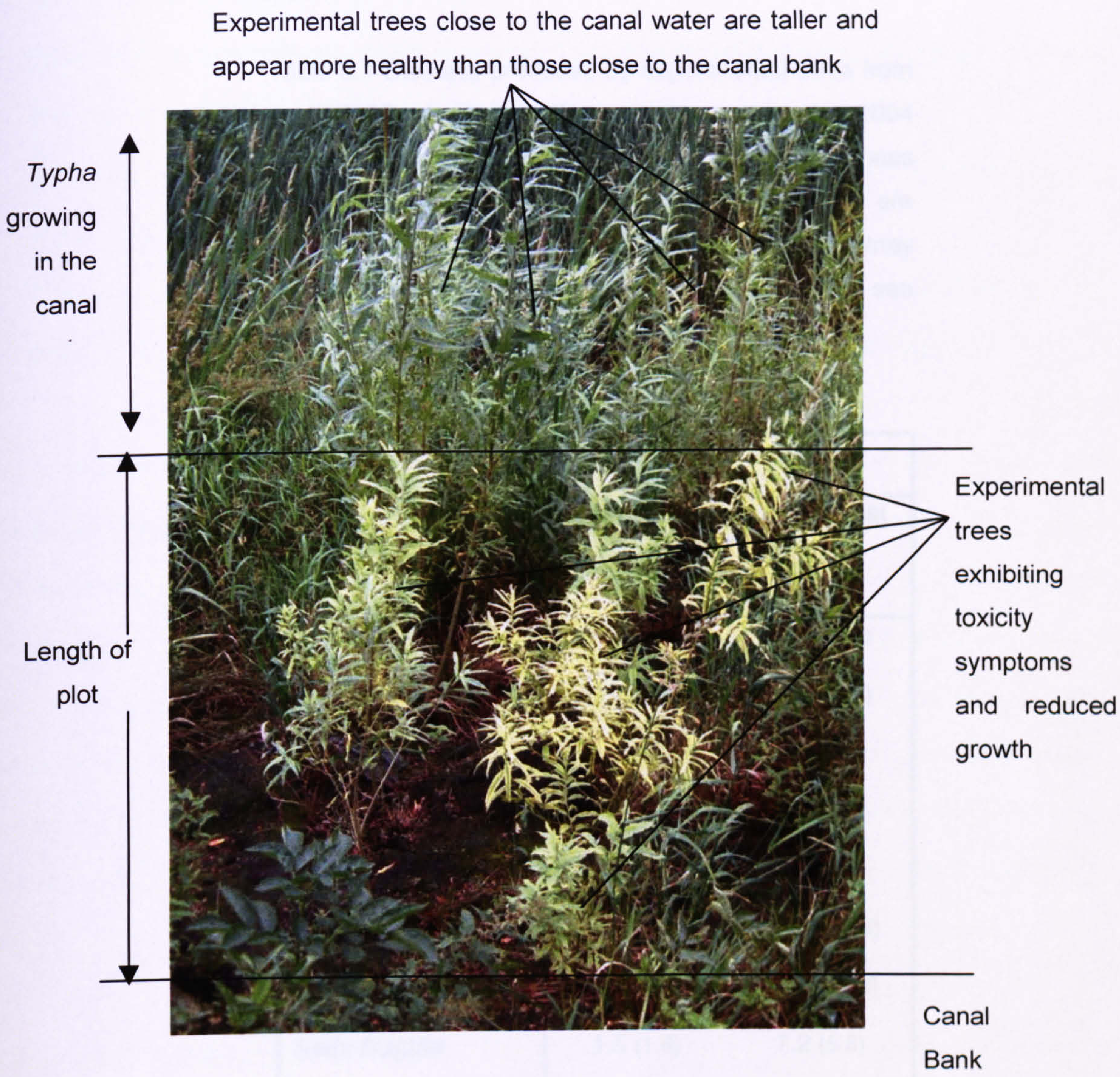
An Investigation of the Factors Affecting Tree Growth at the Woolston New Cut Canal Field Site

8.1 Introduction

Chapter 7 described the set-up and results of the phytoremediation field trial around which the current PhD thesis is based. At the field site, it was noticed as early as the end of the first growing season (2002), that systematic differences in growth were occurring along the length of the canal site. The three factors thought likely to have been causing these differences (sediment moisture content, sediment depth and degree of shading) were tested at two levels each. Three tree species, and also a "no tree" treatments were also included in the design. These factors were tested in all possible combinations.

8.1.1 Differential Growth of Experimental Trees on the Planting Platform

As described in Chapter 7, the set-up of the experimental trees on the WNCC planting platform was such that, on each planted plot, two rows of trees ran from close to the canal bank (trees 1 and 7), to the edge of the canal water (trees 6 and 12) (see Appendix 6). At the end of the 2002 growing season it was noticed that the growth of those trees growing close to the canal bank tended to be stunted, with signs of toxicity (Fig. 8.1) but the trees close to the water appeared healthier. To quantify this phenomenon, plots were divided laterally in half for the measurement of biomass at the canal site i.e. trees 1, 2, 3, 7, 8 and 9 were grouped for biomass representing the trees close to the bank, and trees 4, 5, 6, 10, 11 and 12 were grouped, representing biomass from close to the canal water (Appendix 6). The biomass results obtained from the 2004 harvest (Table 8.1) show that biomass production by trees growing close to the canal bank was consistently lower for all species than by those growing close to the water. To determine what could be causing this difference, observations of the planting platform environment were made.



Experimental trees close to the canal water are taller and appear more healthy than those close to the canal bank

Typha growing in the canal

Length of plot

Experimental trees exhibiting toxicity symptoms and reduced growth

Canal Bank

Figure 8.1 Willow trees at the Woolston New Cut Canal field site exhibiting stunted growth and increased toxicity symptoms close to the canal bank (bottom of the photograph) but appearing healthy close to the canal water (top of the photograph). The photograph was taken from the canal bank by Rosalind King in July 2003.

Table 8.1 Biomass produced by experimental trees from the WNCC planting platform at the end of the 2004 growing season. Values are mean (SD) (n = 6). Biomass production near to the bank and near to the water are significantly different $p < 0.001$, $Z = -4.49$ (Mann-Whitney U test). For the definition of "bank" and "water" see Appendix 6.

	Biomass (t ha^{-1})	
Species	**Near Canal Bank**	**Near Canal Water**
Alnus cordata	2.5 (4.0)	8.2 (10.9)
Alnus glutinosa	3.1 (4.9)	12.6 (9.8)
Alnus incana	1.3 (2.5)	6.8 (6.2)
***Populus* Ghoy**	1.1 (1.9)	2.2 (2.1)
***Populus* Trichobel**	1.4 (1.7)	4.5 (3.4)
***Salix* Ashton Stott**	0.7 (0.9)	15.3 (10.8)
***Salix* Calodendron**	2.3 (2.4)	16.1 (10.6)
Salix fragilis	1.5 (1.6)	7.2 (5.8)
***Salix* Jorrun**	1.3 (2.0)	12.0 (8.6)
***Salix* Sericans**	1.5 (1.7)	6.6 (3.1)
***Salix* Tora**	3.0 (2.0)	13.6 (6.3)

The factors isolated as potential causes were:

- Sediment moisture content – moisture content of sediment close to the canal water was higher than that of sediment close to the canal bank.
- Sediment depth – sediment close to the canal water was noticeably deeper than the sediment close to the canal bank.
- Degree of shading – The hawthorn "hedge" (approximately 5 m high) cast shade on only those trees close to the canal bank during the summer months, when the sun was high.

The differences in sediment depth and moisture content may have been due to the slight topographic gradient that runs from the bank down towards the centre of the canal. This gradient may have led to pooling of the wet sediment when it was originally dredged to form the platform. This effect could explain the increased sediment depth close to the water. The shading was caused by both the large hawthorn hedge and the canal wall. The trees were planted on the south bank of the canal and the hedge was to the south of the site (approximately 4 m from the plot), thus it had a shading effect on the trees, particularly those closest to the hedge (and wall). This effect was compounded close to the wall by the shading effect of the wall itself which is approximately 1 m high.

The following discussion as to the effects of water-stress on plants is mainly based on Leopold and Kriedeman (1975) and references therein, unless otherwise cited. The most obvious and outwardly visible sign of water-stress (due to insufficient water) in plants is wilting. However, even when wilting is not visible, the plant will have already undergone physiological reactions to moisture stress. Transpiration rate is decreased by partial closure of the stomata, and turgor-dependent mechanisms within the plant, such as leaf enlargement, cease. The turgor pressure at which these effects occur is dependent upon species, but leaf enlargement will cease at around -4×10^5 Nm^{-2} and stomata will close between -10 and -15×10^5 Nm^{-2}. Stomatal closure, or partial closure, stops or reduces gaseous diffusion in and out of the leaves. This negatively affects photosynthesis. Water stress has also been shown to affect plant hormone levels. For example, leaf concentrations of ABA (abscisic acid)-like inhibitors increase, leading to stomatal closure. Under more severe drought they may increase to many times their original levels, thus impeding the return to normal function once the period of drought has passed. High levels of these molecules inhibit synthesis of RNA and photosynthetic enzymes. Water stress may also negatively affect root permeability, lessening the plant's ability to restore turgor pressure in the leaves after rewatering of the soil. Due to the disruption of these physiological systems, growth following water stress can be inhibited.

Water stress has been shown to significantly reduce the dry mass of *Salix viminalis* (Martin and Stephens, 2005) and *Pinus radiata* (Watt *et al.*, 2003). Water stressed *Salix viminalis* growing in clay soils apportioned more dry mass to their roots, and less to their stems, than unstressed trees. Stressed trees also showed a decreased ability to utilise available nutrients from the soil. Stress resulted in fewer leaves per shoot and smaller average leaf areas in the *Salix* trees. Water stress induced by competition with weeds was the major factor contributing to growth losses during the first year of *Pinus radiata* growth (Watt *et al.*, 2003). During the second year, growth was mediated through light and water availability.

The following paragraph, discussing the effects of light on plant growth is mainly based on Hart (1988) unless otherwise referenced. Some plant species are specially physiologically adapted to shaded conditions and some species are "shade avoiders". Shade-tolerant species tend to exhibit lower metabolic rates and large, thin leaves. Mechanisms to help plants to "avoid shade" include enhanced axis elongation, increased internode and petiole extension, strong apical dominance and limited leaf development. This effect is highlighted in Figure 8.2 (modified from Hart, 1988). Light has a stimulatory effect on cell division and expansion of leaves. This leads to larger, thicker leaves with higher cell numbers in those leaves exposed to higher levels of PAR (photosynthetically active radiation) with respect to those exposed to lower levels. Phytochrome detects light quality and exists in plants in two photo-convertible states (Pr and Pfr). The former is increased in abundance when the plant is exposed to high levels of far red light (~ 700 – 800 nm). Conversely, the abundance of Pfr is increased by exposure of the plant to high levels of red light (peak

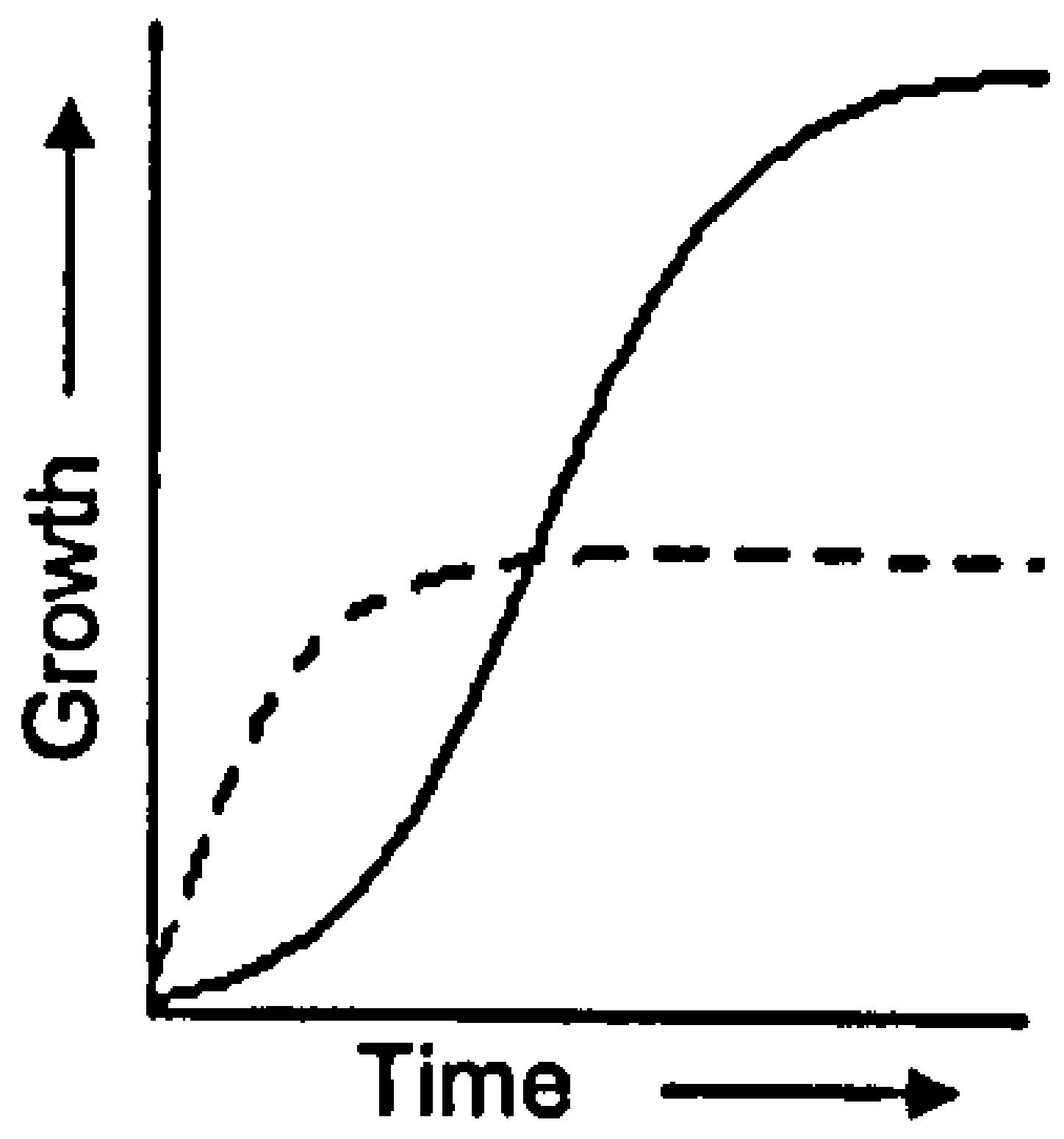


Figure 8.2 Growth of seedlings in constant darkness (solid line) and constant light (dashed line)

absorption 660 nm) (Khattak *et al.*, 2004). High levels of Pfr lead to inhibition of stem elongation. In dense canopies, red light is absorbed more strongly than far red light, which is transmitted down to the lower canopy. Thus, trees in the lower canopy exhibit higher concentrations of Pr, and stem elongation occurs to aid the plant in light capture (Khattak *et al.*, 2004).

Watt *et al.* (2003) studied the effects of shading by weeds on growth of *P. radiata* and found significant increases in stemwood allocation at the expense of roots and shoots in shaded plants. Shading led to a decrease in growth of around 15 % with respect to non-shaded plants. Fownes and Harrington (2004) report that *Betula papyrifera, B. lenta, Pinus strobus, Acer rubrum, Quercus rubra and Tsuga canadensis* all exhibited increasing leaf mass per unit area with increasing light availability.

8.1.2 Aim

- To determine which environmental, chemical or physical factors were affecting tree height and biomass production at the WNCC site.

8.1.3. Hypothesis

That the poor biomass production close to the canal wall was due to the shallow sediment depth, drier conditions and the shading effect of the hedge and the canal wall.

8.2 Methods

The environmental variables observed on the WNCC planting platform were investigated by means of a pot experiment, located at The University of Liverpool Botanical Gardens at Ness, in the NW of England. The three factors tested were:

- High watering regime vs. low watering regime
- Deep sediment vs. shallow sediment
- Shading vs. not shading

The factors were tested together in all combinations using three tree species (an alder, a poplar and a willow) and also a "no tree" treatment (Table 8.2), so that the effect of the trees could be differentiated from that of the individual treatments. Three replicates of each treatment combination were constructed, giving a total of 96 pots. For all combinations used see Table 8.5 and note that the same combinations were also present in the "no tree" treatment. Trees were sourced from the same stock as those planted at the field site. Placement of treatment combinations within the experiment was determined by the use of a fully factorial, randomised block design, as represented in Appendix 9.

8.2.1 Preparation of Treatments

In order to manipulate sediment depth, whilst maintaining the volume of sediment in both the "deep" and "shallow" sediment depth treatments, square white plant pots were ordered with differential rim dimensions (39 cm and 28 cm). It had been intended that the deep treatment be twice as deep as the shallow treatment. However, the pots were differentially tapered towards the base and the difference in sediment depth was not as great as had been intended (Table 8.2). The pots and sediment volume were chosen so as to avoid the trees becoming pot-bound during the experimental period.

Sediment was dredged from the WNCC field site and stored in a lined bund for 50 days prior to use in the current experiment. After thorough mixing, 11.3 L of sediment was added to each of the pots. Each pot had several holes drilled in the bottom to allow free drainage of excess water. Pots were then laid out in their places, as dictated by the random design. Shading was applied to three of the six blocks via specifically constructed shading structures which consisted of a wooden frame with commercial heavy duty orange netting covering the sun-facing side. Strips of heavy-duty black plastic were attached to this orange netting, thus forming dappled shade, akin to that experienced by the trees on the canal planting platform (Fig. 8.3). Care was taken to ensure that no shade extended beyond the boundary of the "shaded" treatments. One tree was planted per pot in the tree-containing treatments. Two different watering regimes were applied via the irrigation pipes visible in Figure 8.3. The volume of water applied to pots with the high watering regime was double

Table 8.2 The factors and treatment levels used. Treatments were used in all combinations with other treatments.

Factor Tested	**Different Treatments**			
Relative Watering Regime	1	0.5		
Sediment Depth (cm)	20	15		
Shading	Yes	No		
Species used *	*Alnus glutinosa*	*Populus* Trichobel	*Salix* Tora	No Tree

* For species names see Royle *et al.* 2003.

Figure 8.3 Set-up of the experimental block design with shade structures. Shaded plots are to the right hand side of the shade structures. Photograph taken by Mike O'Connor.

that of the pots with the low watering regime. The exact volume of water applied to the pots varied throughout the year according to the amount of local rainfall, increasing when rainfall was low and decreasing when rainfall was high. Two separate pipes were used to deliver the different watering regimes. Both were connected to automatic pumps. Pumps were deactivated in the winter when the trees were dormant. The experimental set-up was completed on 6th June 2003.

8.2.2 Sampling and Duration

Initial sediment samples were taken from each pot and bulked by row on 13th June 2003, giving 12 samples in total. Sediment was sampled from every pot to a depth of 10 cm, at 7 months, 12 months and 15 months after this time into polyethylene bags using a trowel. This depth was chosen for comparability with the canal site where 10 cm deep cores were taken.

After a growing period of 21 months, trees were coppiced in March 2005 and final biomass was immediately weighed using a field balance. Representative stem samples were taken from each tree by cutting a variety of sections from the stem and branches of all sizes. These samples were used for determination of heavy metal content. Further stem samples were dried at 60 °C for determination of stem dry weight. Two measurements of tree heights had been made during the 21-month experimental period. PAR (photosynthetically active radiation) was measured five times throughout the experiment at every pot at 1 m height and also at the experimental field site for comparison. An AVO Multimeter (M2035 mV range with a DeltaT Quantum sensor measuring light between wavelengths 400 to 700 nm) was used for PAR measurement.

8.2.3 Analytical Methodology

The pH, conductivity and moisture content of the sediment were determined on subsamples of fresh sediment. The remaining fresh sediment was dried, ground, sieved to < 2mm and stored in polyethylene bags prior to further analysis. The $CaCl_2$ and EDTA extraction, acid digests and the analyses mentioned above were carried out as described in Chapter 7, Sections 7.2.3 to 7.2.5.2.

Standard reference materials were run with all batches of samples as a quality check as described in Chapter 7.

8.2.4 Statistical Analysis

Three and four-way analysis of variance were carried out on much of the data, using the GLM (general linear model). Where appropriate, Kruskall-Wallis (> 2 samples) or Mann-Whitney U tests (2 samples) were used due to the non-parametric nature of the data. Quoted p-values have been derived from these tests.

8.3 Results

8.3.2 Success of Treatments

High vs. Low Water Regime

The high water regime gave rise to a higher average sediment moisture content than did the lower watering regime and also resulted in a higher final sediment pH (Table 8.3). It is likely that the slightly higher pH under the higher moisture regime was due to a more reduced sediment, owing to the presence of water in more of the sediment pore spaces, thus limiting oxygen diffusion into the sediment. Occasional waterlogging of some of the high water regime pots was caused by the deposition of iron oxide crusts blocking the drainage holes. When waterlogging was noticed, holes were unblocked and the pots drained. The mean difference in moisture contents between the treatments was 10 % after 15 months. The 15 month samples were the only set of samples taken during the growing season. The mean difference between the planting platform sediment near the bank and near the canal water at the WNCC site was 16 % and hence the effect of the different moisture regimes in the current experiment may not be as pronounced as at the canal site. However, samples were only ever taken from the canal site after the end of the growing season when local rainfall was increased.

Table 8.3 The effects of the different water regimes on the four sediment properties after 15 months. Values are mean (SD) (n = 48). Statistical significances are derived from Mann-Whitney U tests.

	Treatment		
	High Water Regime	**Low Water Regime**	**p-value**
H_2O content (%)	62.8 (22.4)	52.2 (10.7)	0.018
pH	3.83	3.66	0.007
Conductivity (mS)	0.75 (0.45)	0.88 (0.41)	0.203
LOI (%)	17.4 (7.5)	18.37 (7.84)	0.323

Table 8.4 Biomass production by the three tree species in the pot experiment under the different regimes. Data are shown in bold where significant differences were observed between treatment levels. Statistical significance is indicated by bold numbering and asterisks p < 0.05 (*), p < 0.01 (**), p < 0.001 (***). Values are mean (SD) (n = 42).

	Species biomass (kg tree^{-1})					
Treatment	Alnus	*Sig*	Populus	*Sig*	Salix	*Sig*
High water Regime	**0.072 (0.037)**	**	**0.120 (0.060)**	*	**0.122 (0.030)**	***
Low Water Regime	**0.041 (0.017)**		**0.067 (0.025)**		**0.069 (0.024)**	
Deep Sediment	**0.071 (0.036)**	*	0.108 (0.059)		0.100 (0.042)	
Shallow Sediment	**0.047 (0.028)**		0.082 (0.045)		0.088 (0.035)	
Shaded	0.063 (0.026)		0.111 (0.062)		**0.106 (0.039)**	*
Non-Shaded	0.055 (0.040)		0.077 (0.036)		**0.081 (0.033)**	

Shaded vs. Non-Shaded Treatments, and Sediment Depth

The shade structures proved to be effective, as shaded treatments received significantly less ($p < 0.001$) PAR (0.54 ± 0.26 mmol m^{-1} s^{-1}) than the non-shaded treatments did (1.70 ± 0.11 mmol m^{-1} s-^{1}). Mean PAR close to the bank and close to the water at the WNCC site were 0.30 ± 0.40 and 1.24 ± 0.55 mmol m^{-1} s^{-1} respectively ($n = 78$), when measured at the same time of year, on a day with similar weather. Thus the levels of light were a little higher during the current pot study than at the WNCC site. Measurement of PAR depends very much on the conditions of the day, and readings vary throughout the day. Thus, in reality, a direct, accurate comparison cannot be made of the degree of shading between the sites. Neither the shading treatment nor differential sediment depth, significantly affected any of the measured environmental variables presented in Table 8.3, and therefore they are not shown.

8.3.3 Biomass Production

Over all treatments, *Alnus glutinosa* produced lower biomass on average (0.059 ± 0.033 kg $tree^{-1}$) than did *Populus* Trichobel (0.094 ± 0.053 kg $tree^{-1}$) or *Salix* Tora (0.094 ± 0.038 kg $tree^{-1}$). The high- and low-watering regimes had a significant effect on the biomass of all tree species (Table 8.4) with all species producing higher biomass under wetter conditions. This is in agreement with the findings of Watt *et al.* (2003) and of Martin and Stephens (2005). Indeed, the top 3 yields for all species were under wet conditions (Table 8.5). Sediment depth had a significant effect only on Alder biomass, and shading only on willow biomass (Table 8.4). The increased biomass in the shaded willows is likely to have been due to increased tree height. The final measure of tree heights gave the average height of shaded willows as 193 ± 42 cm and of non-shaded willows as 161 ± 41 cm. However, this effect is not seen at the canal site. This discrepancy suggests that the shading effect of the hawthorn hedge on the canal planting platform is not one of the dominant factors affecting tree height there. Figure 8.1 shows willows close to the canal bank and close to the canal water. Those willows close to the bank do not appear to be exhibiting stem elongation and strong apical dominance, as they would be if the phytochrome photoequilibria were dominated by Pr. Stem elongation would have been expected due to the shaded conditions, whereas height

Table 8.5 Tree biomass produced under each treatment combination. Biomass production has been ranked and the top 3 ranked (1 to 3) are shown as are the two lowest ranked (7 and 8, or 6 and 7 in the case of *Salix* as two values were equal). Ranks reflect mean values and take no account of significant differences. Values are mean (SD), n = 3.

			Biomass (kg tree^{-1})					
Watering Regime	**Sediment Depth**	**Shaded?**	***Alnus***	**Rank**	***Populus***	**Rank**	***Salix***	**Rank**
Low	**Deep**	**No**	0.049 (0.017)		0.076 (0.015)		0.066 (0.033)	
High	**Deep**	**No**	0.110 (0.032)	1	0.099 (0.017)	3	0.089 (0.046)	
Low	**Shallow**	**No**	0.028 (0.008)	7	0.053 (0.041)	8	0.058 (0.014)	7
High	**Shallow**	**No**	0.026 (0.009)	8	0.080 (0.057)		0.134 (0.019)	2
Low	**Deep**	**Yes**	0.041 (0.013)		0.076 (0.009)		0.089 (0.009)	
High	**Deep**	**Yes**	0.078 (0.027)	3	0.171 (0.091)	1	0.153 (0.014)	1
Low	**Shallow**	**Yes**	0.044 (0.033)		0.067 (0.026)	7	0.062 (0.032)	6
High	**Shallow**	**Yes**	0.073 (0.025)	2	0.132 (0.028)	2	0.120 (0.017)	3

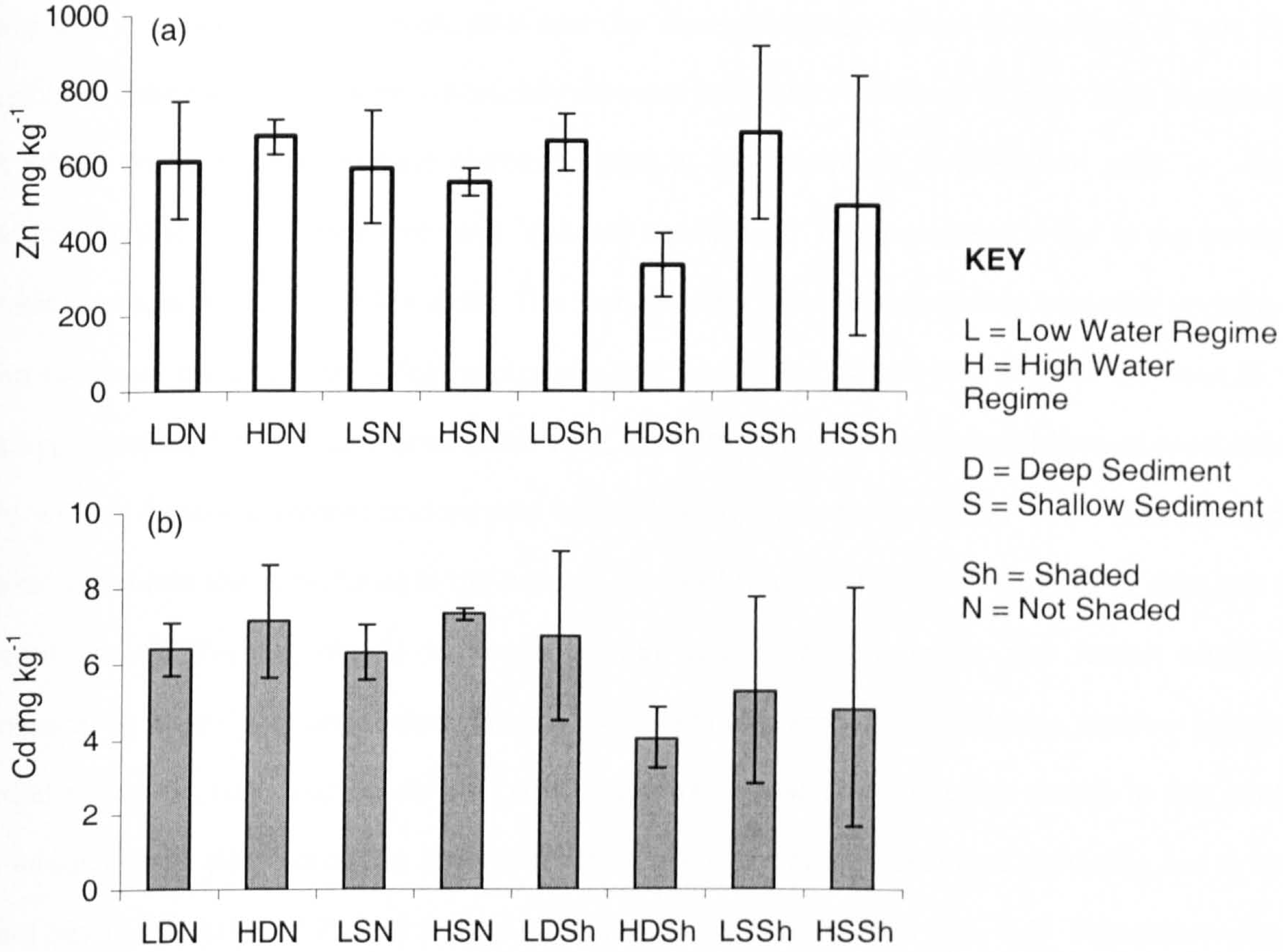


Figure 8.4 Zinc (a) and Cd (b) concentrations after 21 months in the wood of experimental willow trees experiencing different treatment combinations. Values are mean ± SD (n = 3).

stunting would be normally occur in a high light intensity environment. Instead willow growth at the field site appears to have been stunted by a mechanism other than a response to light.

Table 8.5 details tree biomass production by treatment combination. Biomass production in *Alnus glutinosa* was significantly different between treatments ($p = 0.049$, GLM 3-way ANOVA). However, owing to the small number of replicates and the non-parametric nature of the data, it was not possible to determine which were statistically different using Mann-Whitney U tests. Both the poplar and willow produced their highest biomass yields in the treatment combination with a high watering regime, deep sediment and shaded conditions. This combination led to the second highest biomass production in the alder. The highest alder biomass production occurred under the same sediment conditions as willow and poplar, but un-shaded, rather than shaded (Table 8.5). It was hypothesised that the conditions at the WNCC site that yielded the highest biomass production were wetter, deeper sediment and sunnier conditions. Therefore, the results of the current study appear to confirm this hypothesis in the case of *Alnus glutinosa*. The willow and poplar data are in agreement with this hypothesis for water content and sediment depth. The lowest biomass production by willow and poplar was in those treatments with low watering regime, shallow and un-shaded sediment. That shading did not produce the same results at the canal site as in this study has already been discussed. The treatment which led to the highest biomass yield also led to the lowest bioaccumulation of Zn and (mean) Cd in the stems of *Salix* Tora (Fig. 8.4). This pattern was not so evident in poplar or alder stems. Sediment depth and watering regime appear to have had the largest effect on tree biomass production in the current study.

8.3.4 Heavy Metal Behaviour

Lower pH in treatments with the low watering regime (compared to the higher watering regime) may have caused higher mobilisation of heavy metals. There were no significant differences in extractability of metals between any of the tree species, or with the "no tree" treatment, for any of the heavy metals of interest. Thus the data have been grouped, regardless of species. Results of the calcium chloride, EDTA and total metal extractions are presented in Table 8.6. When compared to Figures 7.1 and 7.2, some differences in element content and behaviour are evident. Sediment

Table 8.6 Metal extracted (mg kg^{-1}) from the experimental sediment at the beginning of the experiment "Initial" in June 2003, and at the final sediment sampling time "Final" 15 months later. There were no significant differences between species or in the presence or absence of a tree. Thus the data has been pooled for all pots. For details of extraction processes see Chapter 7, as referenced in section 8.2.3. Loss of total metal is due mostly to leaching loss from the free-draining pots. Values are mean (SD), for "Initial" samples n = 12, for "Final" samples n = 96.

Metal	Time	Extractant		
		$CaCl_2$	EDTA	Acid
Cd	Initial	1.81 (1.01)	2.24 (1.12)	7.50 (1.79)
	Final	0.57 (0.51)	0.32 (0.47)	3.51 (1.46)
Cu	Initial	9.20 (17.3)	110 (26)	395 (112)
	Final	35.1 (14.5)	95 (23)	309 (69)
Pb	Initial	3.96 (5.68)	381 (117)	693 (195)
	Final	19.6 (8.6)	292 (84)	7.5 (166)
Zn	Initial	823 (392)	735 (340)	2116 (691)
	Final	121 (162)	547 (112)	411 (529)

used for the current study had lower initial total pollutant concentrations than the planting platform sediment, which may partly explain the lower incidence of toxicity symptoms in the experimental trees in the current study relative to those at the field site. Zinc toxicity was highlighted as the most likely cause of toxicity symptoms in the experimental trees at the WNCC site (Chapter 7). Initial and final concentrations of total Zn were roughly comparable between the two experiments, though EDTA-extractable Zn was higher in the current study, and $CaCl_2$-extractable Zn lower. Loss of total Zn and Cd occurred over time (due to the free-draining pots) during the current study, as at the canal site. For a discussion of this trend see Chapter 7.

When sediment metals were extracted with $CaCl_2$ at the end of the first growing season, Cd was significantly ($p = 0.050$), and Zn almost significantly ($p = 0.052$), different between high and low water regimes. Cadmium concentration was higher in the low water regime treatment (1.81 ± 0.71 mg kg^{-1}) when compared with the high water regime treatment (1.58 ± 1.03 mg kg^{-1}). The same pattern was evident for the Zn concentrations (421 ± 221 mg kg^{-1} and 333 ± 163 mg kg^{-1} respectively). This may be due to increased oxidation in the low water regime treatments, leading to increased Cd and Zn lability (See the discussion of this in Chapter 3 and Chapter 4).

Fifteen months after establishment of the experiment, total concentrations of Cd and Zn in the sediment exhibited significant differences between the shallow and deep sediment treatments ($p < 0.05$). The shallow sediment treatment retained higher total Cd and Zn concentrations than the deep sediment treatments (Fig. 8.5). There had been no significant difference between these treatments before 15 months and the reason for the difference is unknown. None of the other treatments caused significant differences in total metal concentration.

Calcium chloride extractable concentrations of Cu, Pb and Zn at the sampling time of 15 months were significantly different ($p < 0.05$) between the deep and shallow sediment treatments (Table 8.6). Calcium chloride-extractable Cd concentration was not significantly different between these treatments ($p = 0.096$) due to high variation within the data. Higher mean concentrations of $CaCl_2$-extractable Zn and Cd were present in shallow sediment than the deep sediment treatments. The converse was true for Pb and Cu.

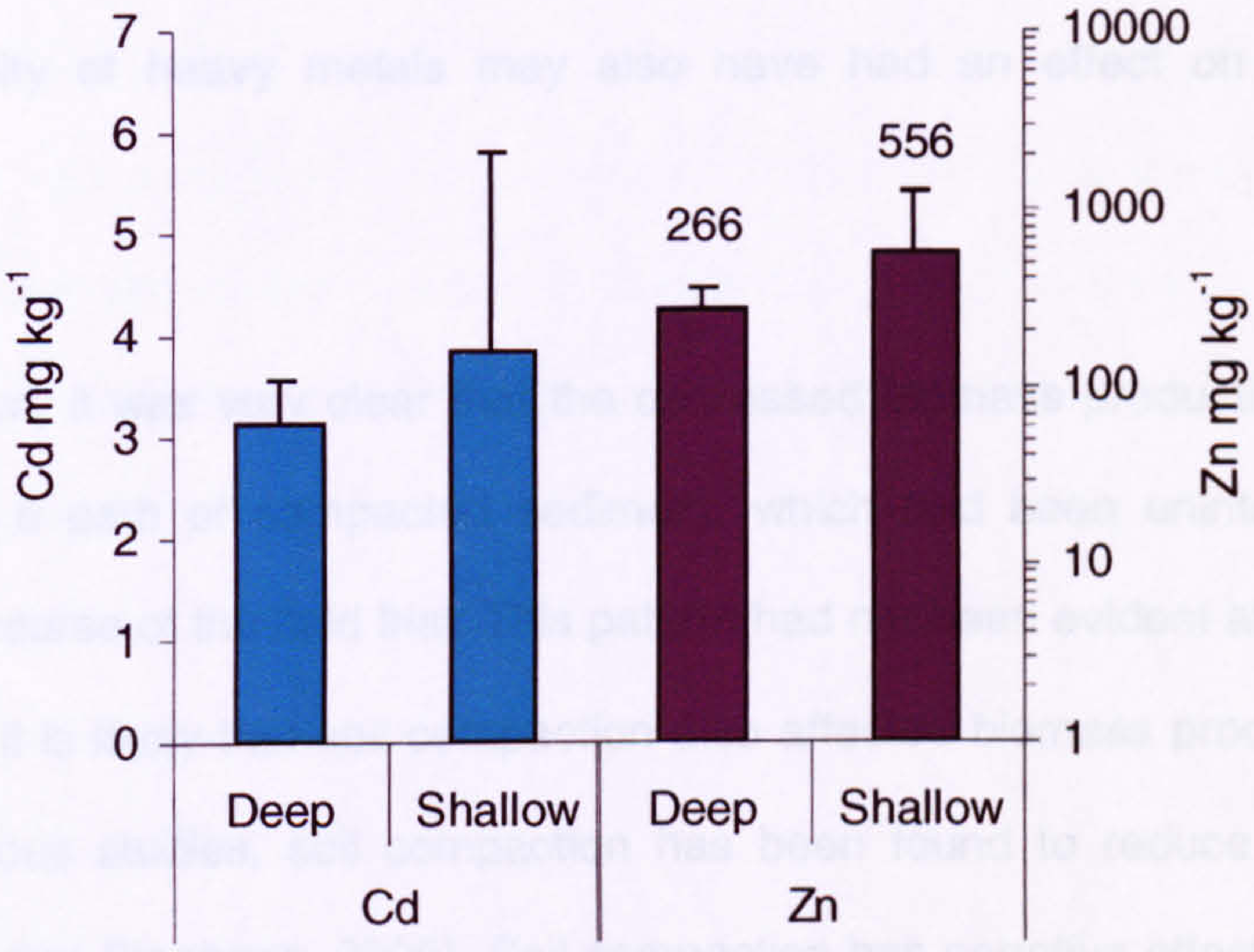


Figure 8.5 Total Cd and Zn concentrations in treatments containing deep and shallow soil at 15 months. Values are mean + SD (n = 48).

No chemical method of measuring heavy metal extractability correlated well with biomass production, and no severe toxicity symptoms were observed in the pot experiment. However, Figure 8.4 shows that heavy metal bioavailability and hence bioaccumulation, were lower in the treatment which produced the highest willow biomass. Also, mean uptake of Cd and Zn into willow stems was lower than uptake rates seen at the canal site (Chapter 7). Uptake of Zn into alder and poplar stems was respectively ~ 9% and 20 % lower in the current experiment than at the canal site (data not shown). Uptake of Cd for the latter two species was comparable between the experiments. Thus, though no extractants accurately predicted metal bioavailability in the current study, Cd and Zn bioavailability of heavy metals may also have had an effect on biomass production.

During the 2004 growing season, it was very clear that the decreased biomass production at the field site was occurring along a path of compacted sediment, which had been unintentionally formed along the site over the course of the field trial. This pattern had not been evident at the start of the current study. However, it is likely that soil compaction also affected biomass production at the experimental site. In previous studies, soil compaction has been found to reduce biomass yields in *Salix viminalis* (Martin and Stephens, 2005). Soil compaction has negative effects on root growth, with roots mechanically impeded by the compacted soil. Reduced root length, increased root diameter and alteration to lateral rooting patterns can adversely affect the function of root systems (Scott *et al.*, 2005).

8.4 Conclusion

The two factors tested in the pot experiment which had the largest effect on biomass production were moisture regime and sediment depth. Lower sediment moisture content and shallower sediment produced the lowest biomass yields in the experimental trees. As well as having the least beneficial physical conditions, these treatments had the highest $CaCl_2$-extractable concentrations of Cd and Zn of their treatment level pairs. Heavy metal toxicity and low sediment pH may have been the major causes of toxicity to the experimental trees both in the pot experiment and at the WNCC canal field site (Chapter 7). Total concentrations of Cd and Zn were higher in the shallow sediment after 15 months. Biomass production at the WNCC field site was also lower in the drier, shallower sediment close to the bank, than in the deeper, wetter sediment close to the canal water. Shade appeared not to be a factor affecting the low biomass production close to the bank at the canal site as, in the current study, shade tended to increase willow biomass production. Treatment combination appeared to affect Cd and Zn bioavailability to *Salix* Tora, with the lowest bioaccumulation occurring in the wetter, deeper, shaded sediment. However, this was not true for *Populus* Trichobel or *Alnus glutinosa.* It can therefore be concluded that, of the factors tested, sediment depth and moisture content had the largest effects on biomass production in this experiment. Biomass production was highest both in the current study, and at the canal site, when the sediment was deeper and had a higher moisture content. Therefore, to increase biomass production near the canal bank and reduce the possible toxic effects of metals, the sediment depth and moisture content should be increased. However, a decrease in heavy metal bioavailability may also reduce the viability of phytoremediation at the site.

CHAPTER 9

General Discussion and Conclusions

The Woolston New Cut Canal sediment is a novel substrate for study. It is high in organic carbon, contains relatively high concentrations of both organic and inorganic pollutants, and undergoes very significant acidification during oxidation owing to the lack of a carbonate mineral phase. Both this acidification and the change in redox potential itself, caused large changes in both the fractionation and the lability of heavy metals within the sediment. There is an almost total lack of literature regarding sediments which readily acidify upon oxidation. Sediments containing significant carbonate phases have been the focus of many studies (Caille *et al.*, 2003, Tack *et al.*, 1996, Tack *et al.*, 1998, Tack *et al.*, 1999), whereas information pertaining to sediments lacking this phase is limited (Gambrell *et al.*, 1991).

The use of an anoxic sequential extraction procedure appeared to approximately estimate Zn lability during oxidation, though not the lability of Cu (Chapters 3 and 4). Labile and NH_4Cl-extractable Zn both underwent massive increases as the sediment became more oxidised. These results suggest that the labile forms of Zn in the oxidising WNCC sediment were also readily extracted by weak extractants such as water or 0.1 M NH_4Cl solution. Zinc is a phytotoxic heavy metal and such high concentrations of labile Zn could pose a significant toxic hazard to species growing in oxidising sediment. Little increase in NH_4Cl-extractable Cu occurred during oxidation, though labile Cu increased very significantly. Therefore, labile forms of Cu are not necessarily extractable with weak extractants, and as a result, a sequential extraction technique alone may underestimate Cu lability in this sediment. Copper is also a phytotoxic metal and to underestimate lability may be to underestimate phytotoxicity of the sediment.

Waterlogging and anoxia may cause terrestrial plant mortality in the sediment when it is first dredged, though available heavy metal concentrations would be very low. This may benefit those flood-tolerant species such as *Salix*, by reducing weed competition. Drying of the sediment, oxidation, decreased pH and increased heavy metal lability led to increased phytotoxicity, especially to the broadleaved species *Brassica napus* in the root elongation assay. *Lolium multiflorum* exhibited different rooting behaviour to *B. napus* making determination of phytotoxicity difficult.

The severe phytotoxicity associated with the oxidised sediment can however be abated via the use of amendment materials. The addition of lime to raise the pH, and thus decrease heavy metal phytoavailability, proved to be very successful in increasing biomass, though phytoaccumulation decreased (Chapter 6). Addition of K_2HPO_4 in combination with lime, reduced the proportion of soil solution heavy metals present as free ions compared to the addition of lime alone. The effect of the extra phosphate addition may have been responsible for improved plant biomass production, and plant uptake of heavy metals was also decreased further.

Addition of amendments to the WNCC would be a possibility to increase the sediment ph and decrease the toxicity of heavy metals. Lime was not an effective amendment at the 1 % level and thus addition of 2.5 % lime would be necessary to control heavy metal mobility. However, this would probably not be an economically viable option on a field scale. Even the addition of lime at the 2.5 % level may not have been sufficient to reduce heavy metal availability in the long-term as, for this treatment, the pore water concentrations of Cd, Cr and Zn post-growth of *Lolium multiflorum* were higher than the pre-growth concentrations, suggesting increased mobilisation over the growing period. The addition of lime at the 5 % level was sufficient that no increase in mobility occurred during the experimental period (Chapter 6). However, addition at this rate would mean total addition of 2000 t of lime to the Woolston New Cut Canal site, were all of the sediment (40 000 t) to be dredged, which would certainly not be economically viable.

The equilibrium concentration (C_e) of heavy metals, as calculated using DGT techniques, correlated well with plant uptake of Cu, Zn, and to a lesser extent Pb. Linear regression showed that this technique could be used to accurately predict uptake of Cu ($r^2 = 0.979$) and Zn ($r^2 = 0.908$) by *Lolium multiflorum* in this sediment, for a range of amendments. The discrepancy between C_e and the actual sediment solution concentrations of heavy metals was evidence for resupply of solution heavy metals from solid-phases. Analysis of flux data proved that this was indeed the case. The addition of the lime and lime/K_2HPO_4 amendments reduced the flux of metals from the sediment solid phases into the solution phases. This reduced not only the solution concentrations of heavy metals, but also the rate at which they could be replenished when depleted. Flux of heavy metals in the WNCC sediment was much higher than soil fluxes reported by Lombi *et al.* (2002). The sediment also reacted differently to the red mud amendment than did the soil studied by Lombi. The red mud amendment, which Lombi had reported as being the most efficient in a near-neutral soil, was the least effective in this acidic sediment. This result highlights the need for further study of acidifying sediments and proves that results reported for soils and pH-buffered sediments are not necessarily transferable to naturally-acidifying sediments.

Under field conditions, the sediment displayed phytotoxicity below what would have been expected from the results of the laboratory studies due to the slower rate of drying (rainfall) and the open system allowing metal leaching. Eight months after dredging, the pH of the sediment at the field-site had dropped to 3.8. As discussed, this decrease in pH will have led to increase availability of heavy metals. Rainfall at the field site appeared to lead to leaching of heavy metals from the sediment to the surrounding canal environment. Of all metals, loss of total concentrations of Zn was most pronounced over the 3-year period, and was perhaps the most significant, due to its phytotoxicity. Such massive losses of total heavy metal concentrations were far above the observed phytoextraction which occurred over the same time period. The data also suggest that little phytostabilisation was occurring. Despite the high concentrations of available heavy metals, phytoextraction was relatively low compared to that reported in the literature (Vandecasteele *et al.*, 2005, Laureysens *et al.*, 2005). Mean biomass production was also low. However, both biomass production and phytoextraction differed significantly between species. *Salix* clones performed best for phytoextraction of Cd, Cu, Zn and Pb, though the *Alnus* species extracted high concentrations of Pb. *Salix* Tora and *Salix* Calodendron both produced high biomass and exhibited high phytoextraction potential compared to the other species. However, *Salix* Tora displayed a higher potential for food chain transfer of heavy metals than *Salix* Calodendron due to higher leaf metal concentrations. Thus, of those tested, Calodendron would be the recommended SRC clone. The results presented in Chapter 6 suggest that addition of an amendment to reduce the acidity of the sediment would not only reduce loss of heavy metals through leaching (and thus reduce environmental pollution), but may also increase biomass production at the experimental site. This may increase the viability of phytoremediation of the site. However, a balance would have to be reached between reduction of phytotoxicity and heavy metal leaching, and phytoavailability for phytoextraction.

Differential growth of trees at the canal site led to an investigation into factors affecting biomass production, and thus phytoremediation, at the site. The pot experiment in which moisture regime, sediment depth and shading were investigated is presented in Chapter 8. The pot experiment appeared to fairly well model heavy metal behaviour at the canal site in terms of loss of total heavy metal content, acidification of the sediment and differential biomass production. The results highlight the need for control of physical as well as chemical conditions during phytoremediation to optimise biomass production and phytoextraction. Shading of the experimental trees at the canal site did not produce the expected stem elongation (which did occur during the pot experiment). This suggested that shade was not a dominant factor affecting tree biomass at the field site. Sediment depth and moisture content appeared to be having the dominant effects on biomass production, both at the field site and in the pot experiment. Higher biomass production occurred in wetter, deeper sediments than in shallower, drier sediments. Coupled with the physical conditions, the wetter, deeper sediment exhibited lower heavy metal availability and a higher pH. Thus, physical and chemical sediment properties were not independent of each other and must be considered in conjunction when managing the sediment. Continued dredging and planting of the sediment in the manner described in Chapter 7 and Royle *et al.* (2003), without further management or treatment, would not be recommended owing to the inability of the current phytoremediation scheme to control leaching losses of heavy metals from the site.

Phytoremediation of the WNCC sediment also included the phytodegradation of organic pollutants (TPH and PAH). Total concentrations of TPH in the field-site sediment decreased over the 3-year time period, though levels of PAH did not. This was possibly due to an interaction between the TPH and the PAH, reducing the bioavailability of the PAH and thus its degradation. The presence of TPH in the sediment may also have affected heavy metal bioavailability in a similar manner. It was more difficult to re-wet dry sediment from the WNCC, than from The University of Liverpool Botanic

Gardens at Ness. This was most probably due to the sorption of hydrophobic compounds onto sediment particles during drying (Jonker *et al.*, 2003). If organic matter and other sediment exchange sites were occupied by organic contaminants, then the number of heavy metal binding sites would be reduced. Thus, the dual contamination of this sediment may alter its metal sorbing properties with respect to sediments free of organic contamination. Once again, this would make the sediment a novel subject for study. Other reported methods of remediation of organic contaminant pollution such as composting (Semple *et al.*, 2001) and landfarming (Huang *et al.*, 2004) would be faster and more effective treatments for the organic contamination in this sediment than the phytoremediation scheme employed in the current study.

In conclusion, oxidation of the anoxic sediment led to a decrease in pH and large increases in heavy metal mobility and lability. This in turn led to high phytotoxicity and heavy metal leaching potential. Leaching under field conditions led to significant decreases in total heavy metal concentrations and thus most probably to the pollution of the surrounding environment. Extraction of metals by trees tended to be lower than that reported in the literature, but could possibly be improved by manipulating the physical and chemical aspects of the sediment at the site. Dredging of the sediment would not be recommended unless control of pH and heavy metal lability upon oxidation could be controlled and effectively managed in the long term. Features already discussed make this sediment a novel substrate for study.

The data presented in this thesis show that dredging of the relatively inert anoxic sediment caused immobilised heavy metals (bound as sulphides) to mobilise. The pH of the sediment was significantly decreased, further increasing the problem of mobile heavy metals. Leaching led to loss of heavy metals form the sediment into the canal water, and thus pollution of this medium. The amendments tested proved to be ineffective at economically viable addition rates. Owing to the low economic value of the site and the lack of a pathway for the metals in the anoxic, submerged sediment to affect human receptors, the best option for the site at the current moment would be to leave it as a wildlife corridor. For as long as the sediment is submerged it poses minimum risk to human health or ecosystem health. It would therefore be recommended to ensure that there is at least 1 m depth of water in the canal channel at all times so that the sediment is continuously submerged. The canal currently contains many species including tree species, reeds, grasses, rushes, frogs, water fowl and small rodents as well as invertebrates. Any large-scale remediation strategy would have to cause significant damage to the ecosystem in order to try to remediate the sediment, only to try and re-store the ecosystem afterwards. Considering the lack of the pathway link when the sediment is submerged, the cost and benefits of this kind of work are not obvious. Therefore the recommended course of action in this case is the continued submergence of the sediment.

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APPENDIX 1

Concentration of elements and contaminants in the Woolston New Cut Canal sediment as reported by three UKAS accredited laboratories.

	Median				Max				Min		
	Alcontrol	NRM	CAS		Alcontrol	NRM	CAS		Alcontrol	NRM	CAS
PH	5.6	-	-		5.9	0	0		5.1	0	0
solids (air dried) %	54.6	-	-		70.3	-	-		50.5	-	-
stones (>10mm) %	18.4	-	-		35.2	-	-		0	-	-
Conductivity (20°C) µS/cm	1600	1770	2300		1870	2000	2800		1400	1510	1700
Sulphate % SO_4/SO_3	0.52	-	0.75		0.58	-	1.5		0.49	-	0.61
Total Sulphate	-	6105	-		-	6580	-		-	5174	-
Sulphide	430	262	48		4840	769	240		34.9	102	44
Cyanide (total)	97.6	10	18		130	15	43		77	4	4.9
Arsenic	330	766.2	490		472	853.4	540		249	477.2	240
Cadmium	12.9	14.03	19		14.1	16.81	23		10.7	9.66	12
Chromium	807	1497	980		845	1599.1	1000		676	1277.9	930
Chromium (VI)	-	-	29		-	-	32		-	-	18
Lead	1240	2125.4	1500		1300	2385.4	1600		1030	1920.3	1200
Mercury	3.47	7.82	4.8		4.78	8.2	6.7		3.14	6.24	3.3
Selenium	2.21	0.85	0.72		3.02	0.9	1.2		0.74	0.48	0.34

	Median				Max				Min		
	Alcontrol	NRM	CAS		Alcontrol	NRM	CAS		Alcontrol	NRM	CAS
Copper	554	1050.9	720		635	1241.6	890		493	981.5	590
Nickel	65.6	78.2	79		74.8	85	110		60.8	70.5	64
Zinc	3590	6174.4	4700		4130	7394	5300		2980	3815.4	2900
Molybdenum	6.6	6.6	7.3		8.01	9.2	13		3.75	5.8	6.8
Cobalt	-	26.8	26		-	34.3	35		-	25.3	21
Boron	64.7	3.5	1.6		97.9	4.1	2.2		50.7	3.2	1.1
Mineral Oil	8300	-	-		9560	-	-		2920	-	-
Toluene	-	213.3	-		-	261.5	-		-	178.4	-
Naphthalene	275	-	-		275	-	-		275	-	-
TPH (C6-C10)	-	-	-		-	-	-		-	-	-
TPH (C10-C20)	-	-	630		-	-	750		-	-	69
TPH (C20-C40)	-	-	2100		-	-	2600		-	-	300
TPH (C6-C40)	-	-	2700		-	-	3300		-	-	370
TPH	7410	5422	-		8710	6794	-		6510	5138	-
Diesel range organics	3860	-	-		4410	-	-		3530	-	-

	Median				Max				Min		
	Alcontrol	NRM	CAS		Alcontrol	NRM	CAS		Alcontrol	NRM	CAS
Lubricating range organics	3730	-	-		4310	-	-		2780	-	-
Naphthalene	3.01	3.68	-		4.05	4.22	-		1.33	2.79	-
Acenaphthylene	0.53	1	-		0.53	1.14	-		0.53	0.81	-
Acenaphthene	5.51	3.3	-		7.49	5.15	-		4.47	2.78	-
Fluorene	7.43	4.58	-		9.21	6.97	-		5.65	4.08	-
Phenanthrene	14.8	11.87	-		18.3	13.22	-		12.2	8.07	-
Anthracene	11.2	8.83	-		12.1	10.34	-		8.24	7.75	-
Chrysene	18.1	11.18	-		21.5	12.12	-		13.1	8.71	-
Flouranthene	41.7	23.02	-		47.1	26.45	-		37.7	17.99	-
Pyrene	37.9	19.75	-		46.4	22	-		31.9	14.73	-
Benzo (a) anthracene	29.2	9.4	-		36.5	10.32	-		13	7	-
Benzo (b) fluoranthene	-	6.85	-		-	7.43	-		-	5.24	-
Benzo (k) fluoranthene	-	6.31	-		-	6.96	-		-	5.01	-
Benzo (b+k) fluoranthene	17.7	13.09	-		21.8	14.39	-		11.9	10.25	-
Benzo (a) pyrene	13.7	7.33	-		15.6	8.38	-		12.4	5.16	-

	Median				Max				Min		
	Alcontrol	NRM	CAS		Alcontrol	NRM	CAS		Alcontrol	NRM	CAS
Indeno (1,2,3-cd) pyrene	7.13	5.85	-		8.57	6.46	-		5.65	4.35	-
Dibenze (a,h) anthracene	1.365	1.81	-		2.29	1.99	-		0.8	1.28	-
Benzo (g,h,i) perylene	7.88	4.9	-		9.81	5.43	-		6.09	3.65	-
PAH	214	-	120		252	-	140		177	-	98
PAH EPA16	-	130	-		-	144	-		-	99	-
PCB 28 ppb	12	-	-		32	-	-		8.72	-	-
PCB 52 ppb	28	-	-		33.6	-	-		21.2	-	-
PCB 101 ppb	19.1	-	-		21	-	-		16.8	-	-
PCB 118 ppb	4.19	-	-		6.92	-	-		0	-	-
PCB 138 ppb	9.16	-	-		13	-	-		8.04	-	-
PCB 153 ppb	9.16	-	-		11.9	-	-		8.1	-	-
PCB 180 ppb	7.02	-	-		9.66	-	-		5.67	-	-
Total PCB ppb	87	-	-		112	-	-		77	-	-
TEM	-	14080	-		-	15260	-		-	13120	-
Phenols (monhydric)	-	5.4	-		-	7.4	-		-	4.2	-

	Median				Max				Min		
	Alcontrol	NRM	CAS		Alcontrol	NRM	CAS		Alcontrol	NRM	CAS
Total Phenols	-	-	1.4		-	-	1.6		-	-	0.83

APPENDIX 2

Mean values and relative standard deviations (RSD) for heavy metal concentrations in the sequential extraction samples (Chapter 3).

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
Cd	**Mean**	**NH_4Cl**	**0.00**	**0.00**	**0.00**	**0.24**	**1.28**	**14.18**	**30.35**	**42.80**	**15.69**	**5.05**	**30.18**	**3.86**
		NaOAc	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.75**	**0.00**	**0.00**	**1.90**	**2.85**	**3.88**
		Dithionite	**6.00**	**5.97**	**6.30**	**5.25**	**7.25**	**8.47**	**8.19**	**8.05**	**8.00**	**7.50**	**8.05**	**9.61**
		HNO_3/H_2O_2	**8.86**	**8.53**	**10.49**	**7.56**	**8.00**	**7.45**	**8.80**	**12.26**	**10.39**	**12.72**	**7.48**	**7.16**
	RSD	NH_4Cl	-	-	-	200.0	145.8	126.5	92.0	80.8	150.5	152.0	103.6	23.4
		NaOAc	-	-	-	-	-	-	244.9	-	-	200.0	200.0	41.9
		Dithionite	0.0	12.6	15.1	3.9	19.2	13.1	27.7	17.9	19.8	10.9	4.1	26.4
		HNO_3/H_2O_2	8.4	7.9	18.4	4.6	-	7.9	58.1	41.8	29.4	15.6	50.3	9.7
Cr	**Mean**	**NH_4Cl**	**0.38**	**0.67**	**0.28**	**0.23**	**4.45**	**1.70**	**4.41**	**1.06**	**0.67**	**0.97**	**1.15**	**0.52**
		NaOAc	**8.99**	**15.27**	**18.32**	**16.73**	**19.38**	**16.28**	**17.17**	**17.76**	**16.84**	**24.04**	**32.25**	**27.99**
		Dithionite	**364.7**	**366.8**	**384.4**	**373.7**	**401.8**	**484.4**	**476.0**	**469.5**	**519.1**	**494.8**	**501.6**	**517.2**
		HNO_3/H_2O_2	**327.9**	**287.8**	**295.1**	**294.9**	**331.9**	**342.7**	**373.4**	**388.4**	**375.1**	**344.4**	**349.4**	**334.6**
	RSD	NH_4Cl	88.3	129.7	173.2	200.0	108.3	86.2	137.0	2.2	86.6	19.3	4.1	102.3
		NaOAc	10.3	3.2	1.8	22.2	29.1	13.1	13.0	11.4	30.8	24.3	8.4	22.9
		Dithionite	0.0	5.8	2.6	8.4	3.4	5.0	6.8	5.7	6.6	9.6	3.0	3.5
		HNO_3/H_2O_2	8.6	9.0	3.3	16.9	7.8	7.7	14.4	7.9	8.2	12.0	6.8	2.1

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
Cu	**Mean**	**NH_4Cl**	**0.00**	**0.94**	**0.88**	**0.94**	**2.55**	**0.38**	**12.75**	**5.89**	**1.01**	**0.19**	**19.92**	**3.80**
		NaOAc	**0.00**	**0.00**	**0.00**	**0.00**	**4.41**	**5.55**	**19.84**	**13.17**	**14.25**	**0.86**	**34.23**	**15.77**
		Dithionite	**51.00**	**49.06**	**54.55**	**49.68**	**61.52**	**48.41**	**55.75**	**44.14**	**59.64**	**48.00**	**57.13**	**56.96**
		HNO_3/H_2O_2	**397.07**	**410.67**	**437.17**	**401.07**	**479.91**	**484.39**	**437.90**	**552.55**	**509.59**	**522.39**	**446.05**	**448.68**
	RSD	NH_4Cl	-	83.2	173.2	143.3	107.5	115.5	155.0	167.8	173.2	200.0	176.9	96.4
		NaOAc	-	-	-	-	200.0	151.7	126.2	130.1	117.2	200.0	94.0	51.9
		Dithionite	0.0	8.6	20.0	11.3	8.2	14.9	28.2	19.9	11.8	17.4	29.0	16.6
		HNO_3/H_2O_2	8.0	12.1	10.1	7.4	5.0	7.3	19.1	14.7	2.6	9.8	28.2	2.4
Fe	**Mean**	**NH_4Cl**	**3071**	**1815**	**1836**	**2020**	**1159**	**1109**	**1039**	**597**	**493**	**1464**	**440**	**595**
		NaOAc	**1184**	**2278**	**1371**	**1202**	**860**	**818**	**667**	**614**	**556**	**793**	**1012**	**1061**
		Dithionite	**21270**	**21003**	**21818**	**20990**	**26341**	**22885**	**23093**	**21121**	**20528**	**19723**	**21937**	**29959**
		HNO_3/H_2O_2	**11618**	**12657**	**12595**	**13074**	**13810**	**14205**	**14897**	**15778**	**16046**	**14442**	**14071**	**14873**
	RSD	NH_4Cl	9.3	23.9	34.9	27.0	47.1	52.6	85.8	45.9	57.9	56.9	74.2	61.9
		NaOAc	13.1	25.6	36.1	41.9	31.9	69.0	19.0	16.4	40.6	15.9	7.3	24.8
		Dithionite	0.0	10.4	8.7	4.6	11.6	11.0	23.5	7.6	11.9	4.9	6.8	23.3
		HNO_3/H_2O_2	9.4	4.1	6.8	15.9	0.8	7.8	10.0	9.0	11.9	5.1	6.4	5.7

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
Pb	**Mean**	**NH_4Cl**	9.88	7.60	6.30	6.84	14.80	22.54	33.72	50.02	30.35	27.61	81.42	53.36
		NaOAc	105.0	187.4	243.6	201.0	224.9	253.5	298.8	341.7	337.6	329.3	441.8	395.4
		Dithionite	105.0	87.6	120.6	101.1	111.1	117.3	119.7	110.0	148.5	167.3	126.5	105.4
		HNO_3/H_2O_2	536.8	492.7	539.7	489.3	591.6	600.1	651.5	765.8	927.0	1165.5	607.0	650.9
	RSD	NH_4Cl	22.2	89.0	38.9	72.5	108.7	86.4	93.3	41.7	20.6	99.1	50.8	10.2
		NaOAc	18.3	17.8	24.6	18.4	37.8	33.6	17.6	6.8	25.9	18.4	9.8	14.1
		Dithionite	0.0	15.0	10.3	12.1	10.6	10.2	23.1	25.9	9.5	69.4	26.0	15.8
		HNO_3/H_2O_2	6.4	15.3	13.3	8.5	14.2	21.4	28.9	9.7	35.5	73.9	18.6	7.2
Zn	**Mean**	**NH_4Cl**	68.1	62.8	324.8	289.1	344.2	459.5	432.0	1062.3	922.9	441.4	1179.2	1359.4
		NaOAc	90.1	193.8	269.4	232.7	211.2	170.1	179.3	179.9	217.3	274.4	221.0	267.2
		Dithionite	211.0	210.0	185.7	222.1	225.6	214.6	182.3	172.3	265.3	247.2	173.1	191.7
		HNO_3/H_2O_2	1720	1615	1523	1145	1322	1238	1145	2164	1439	1717	1076	786
	RSD	NH_4Cl	19.8	59.9	81.1	83.2	68.5	77.1	60.7	52.8	18.6	56.1	28.1	19.4
		NaOAc	8.0	19.6	30.4	20.4	24.8	13.6	50.3	25.0	20.8	20.6	47.7	26.7
		Dithionite	0.0	12.8	2.4	7.3	28.3	40.7	31.4	14.6	54.5	24.1	27.3	19.9
		HNO_3/H_2O_2	10.0	10.0	16.3	14.7	39.3	44.7	49.1	93.8	60.4	19.6	91.9	9.4

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3
Cd	Mean	NH_4Cl	0.00	0.26	0.49	0.24	0.62	1.00	0.90	0.00	4.57	4.91	0.82	1.69
		NaOAc	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92
		Dithionite	6.00	4.61	5.40	5.00	5.61	7.49	7.20	6.77	6.22	6.78	8.08	7.45
		HNO_3/H_2O_2	8.86	8.64	10.33	11.27	12.58	11.74	13.64	13.76	16.92	14.38	10.79	15.91
	RSD	NH_4Cl	-	200.0	115.5	200.0	115.7	66.7	77.5	-	173.2	121.8	141.4	173.2
		NaOAc	-	-	-	-	-	-	-	-	-	-	-	130.7
		Dithionite	0.0	23.4	4.9	4.4	18.7	15.3	5.2	17.0	18.7	20.7	3.1	24.2
		HNO_3/H_2O_2	8.4	13.1	10.0	42.4	-	-	7.8	17.0	36.2	11.3	32.0	34.5
Cr	Mean	NH_4Cl	0.38	1.13	0.00	0.00	4.09	3.21	11.00	3.70	5.76	12.49	4.53	0.16
		NaOAc	8.99	16.67	19.56	17.83	19.51	14.41	13.83	14.24	19.41	19.94	28.80	21.43
		Dithionite	364.7	367.8	375.5	359.0	356.9	483.1	464.8	475.1	465.3	488.4	518.4	488.1
		HNO_3/H_2O_2	327.9	298.0	319.8	294.6	333.2	351.2	380.8	431.3	374.8	363.6	362.5	374.7
	RSD	NH_4Cl	88.3	120.0	-	-	136.3	102.2	55.9	88.3	41.0	24.2	103.8	173.2
		NaOAc	10.3	11.5	10.3	20.2	12.9	16.1	5.2	1.6	23.8	15.2	17.8	26.7
		Dithionite	0.0	8.1	5.6	4.0	11.3	3.3	2.7	7.7	6.3	12.2	2.5	4.8
		HNO_3/H_2O_2	8.6	7.6	6.9	10.2	7.0	12.9	14.0	3.6	9.3	5.7	3.8	0.9

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3
Cu	Mean	NH_4Cl	0.00	0.95	0.23	0.44	2.22	1.16	5.36	1.52	1.27	8.29	2.09	0.84
		NaOAc	0.00	0.00	0.00	4.67	1.11	0.00	0.00	0.00	0.00	0.00	2.74	7.30
		Dithionite	44.00	41.78	65.46	43.69	47.93	44.38	44.23	42.67	44.79	54.00	60.17	51.79
		HNO_3/H_2O_2	397.07	408.04	451.08	454.87	538.94	470.39	477.43	546.03	541.60	494.08	506.51	617.27
	RSD	NH_4Cl	-	81.5	200.0	115.5	168.1	121.5	101.6	129.7	123.2	43.2	77.4	123.8
		NaOAc	-	-	-	200.0	200.0	-	-	-	-	-	141.4	159.1
		Dithionite	0.0	25.4	19.7	8.0	8.9	9.3	5.8	15.2	13.7	16.5	21.4	13.6
		HNO_3/H_2O_2	8.0	5.8	4.0	19.2	19.4	9.2	12.2	4.3	3.2	7.4	6.3	18.1
Fe	Mean	NH_4Cl	3071	1931	2878	2637	1739	1863	2195	1609	1819	2210	1155	628
		NaOAc	1184	2333	1962	1646	1623	1166	2110	1282	2418	3077	2111	1541
		Dithionite	21270	17610	20891	19041	19950	21658	19363	19230	18010	17529	22242	22748
		HNO_3/H_2O_2	11618	12800	13740	13460	14927	14628	16198	16321	15670	14872	14662	15120
	RSD	NH_4Cl	9.3	24.2	21.6	45.8	35.8	20.3	32.2	6.6	19.0	11.3	96.7	115.0
		NaOAc	13.1	17.6	12.1	24.3	75.0	36.5	48.7	15.2	39.6	14.7	67.4	52.0
		Dithionite	0.0	10.1	6.7	9.4	13.6	13.9	9.1	12.2	7.3	18.1	13.4	14.2
		HNO_3/H_2O_2	9.4	7.7	5.0	8.6	6.9	8.6	15.7	0.8	5.8	6.6	1.6	9.6

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3	1-3
Pb	**Mean**	**NH_4Cl**	**9.88**	**6.73**	**2.99**	**2.20**	**10.78**	**8.10**	**9.60**	**3.61**	**4.31**	**12.34**	**20.72**	**6.59**
		NaOAc	**105.0**	**203.1**	**176.4**	**161.7**	**194.3**	**216.1**	**147.4**	**206.7**	**177.7**	**192.8**	**383.1**	**366.9**
		Dithionite	**105.0**	**93.5**	**119.2**	**94.7**	**89.4**	**108.9**	**130.4**	**119.5**	**123.6**	**138.0**	**138.0**	**100.2**
		HNO_3/H_2O_2	**536.8**	**509.2**	**554.9**	**580.2**	**645.1**	**658.2**	**788.1**	**1080.3**	**874.7**	**909.6**	**804.1**	**802.2**
	RSD	NH_4Cl	22.2	92.2	159.9	103.9	78.5	52.4	72.5	63.1	53.6	36.1	92.9	100.3
		NaOAc	18.3	30.6	11.0	36.1	49.5	42.5	39.2	22.0	16.7	36.1	6.0	30.3
		Dithionite	0.0	13.1	10.9	24.9	10.4	11.8	16.8	12.9	18.8	21.8	16.5	39.4
		HNO_3/H_2O_2	6.4	11.1	4.6	7.1	21.3	36.3	30.0	19.7	4.0	7.4	26.7	14.9
Zn	**Mean**	**NH_4Cl**	**68.1**	**28.5**	**56.5**	**135.0**	**234.6**	**201.8**	**97.8**	**264.5**	**118.2**	**44.8**	**340.1**	**726.1**
		NaOAc	**90.1**	**182.8**	**160.0**	**216.1**	**167.8**	**166.7**	**136.0**	**236.0**	**270.2**	**154.9**	**267.1**	**445.4**
		Dithionite	**211.0**	**235.7**	**180.2**	**238.9**	**195.8**	**244.5**	**226.3**	**231.6**	**284.7**	**266.9**	**188.5**	**269.8**
		HNO_3/H_2O_2	**1720**	**1708**	**2091**	**1887**	**1599**	**1780**	**2017**	**2353**	**2325**	**2357**	**1623**	**2362**
	RSD	NH_4Cl	19.8	33.7	26.7	85.7	81.2	63.6	63.6	37.6	85.4	38.0	122.2	97.9
		NaOAc	8.0	17.4	21.4	40.2	34.6	5.0	43.2	18.9	27.2	10.9	2.2	34.1
		Dithionite	0.0	22.8	17.1	22.7	17.9	38.5	12.0	15.3	17.0	24.5	33.3	6.2
		HNO_3/H_2O_2	10.0	24.5	29.3	31.0	36.9	44.8	19.4	30.2	12.8	9.8	50.5	40.8

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x
Cd	**Mean**	**NH_4Cl**	0.00	0.00	0.00	0.48	0.60	0.63	8.22	0.71	5.14	0.64	21.33	1.59
		NaOAc	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.09	2.59
		Dithionite	6.00	5.66	5.99	5.17	5.11	6.77	7.10	7.45	6.59	6.19	7.46	7.80
		HNO_3/H_2O_2	8.86	9.08	9.75	10.34	13.94	10.00	14.56	14.91	16.31	12.88	9.90	8.67
	RSD	NH_4Cl	-	-	-	115.5	115.8	115.5	179.5	115.5	135.8	115.7	153.2	89.5
		NaOAc	-	-	-	-	-	-	-	-	-	-	200.0	102.5
		Dithionite	0.0	2.3	14.3	1.5	26.4	31.7	7.2	28.0	6.9	9.5	15.5	18.2
		HNO_3/H_2O_2	8.4	6.0	5.5	6.6	-	-	-	5.3	45.7	18.0	55.4	29.8
Cr	**Mean**	**NH_4Cl**	0.38	0.69	0.22	0.21	7.95	4.66	5.82	11.36	4.02	17.69	6.69	3.18
		NaOAc	8.99	19.05	19.11	19.25	17.28	15.14	15.18	14.30	19.98	23.00	27.75	18.61
		Dithionite	364.7	409.9	381.6	386.1	362.9	467.7	455.3	430.4	497.2	490.0	508.4	465.3
		HNO_3/H_2O_2	327.9	310.8	309.9	331.9	337.2	327.8	384.1	426.3	370.3	361.0	369.0	374.9
	RSD	NH_4Cl	88.3	126.6	200.0	200.0	84.3	133.2	60.3	56.9	28.5	44.8	154.5	99.2
		NaOAc	10.3	10.6	0.5	19.1	3.6	20.8	16.7	1.4	11.8	11.4	11.4	24.4
		Dithionite	0.0	6.5	4.9	15.3	2.1	5.3	8.7	8.0	8.4	4.2	4.3	22.2
		HNO_3/H_2O_2	8.6	3.1	3.4	1.6	9.5	2.0	11.0	3.6	5.4	7.2	4.0	9.3

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x
Cu	Mean	NH_4Cl	0.00	0.97	0.46	1.11	4.04	2.06	5.33	8.53	0.76	10.41	9.33	3.62
		NaOAc	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.49	0.00	39.07	20.59
		Dithionite	48.40	49.83	60.67	50.16	56.00	51.39	45.04	38.04	44.25	49.89	61.02	54.49
		HNO_3/H_2O_2	397.07	444.19	429.23	468.17	527.81	465.29	512.78	554.64	523.01	485.46	438.12	469.33
	RSD	NH_4Cl	-	142.6	115.5	120.4	100.2	177.3	98.7	106.1	120.1	60.1	57.2	79.3
		NaOAc	-	-	-	-	-	-	-	-	173.2	-	116.1	153.3
		Dithionite	0.0	12.4	9.1	19.2	7.6	8.4	10.3	11.2	19.5	15.4	7.6	31.5
		HNO_3/H_2O_2	8.0	3.9	2.9	3.0	3.6	5.6	13.1	1.9	19.3	10.1	6.6	7.4
Fe	Mean	NH_4Cl	3071	2671	2544	3609	2186	1987	1771	2422	2136	2221	1018	1015
		NaOAc	1184	2629	2558	1855	2349	2209	2233	2813	3506	3809	2440	1929
		Dithionite	21270	21598	19802	19748	19262	21098	18163	17728	16779	15645	19243	24012
		HNO_3/H_2O_2	11618	13893	13696	14544	14602	14085	18478	17060	14661	14381	13917	14865
	RSD	NH_4Cl	9.3	21.2	24.8	51.1	15.1	32.5	28.6	19.3	33.3	38.7	162.4	96.9
		NaOAc	13.1	21.8	24.0	15.4	41.1	45.8	71.5	35.6	61.4	25.0	70.3	63.8
		Dithionite	0.0	7.5	15.1	21.5	17.4	22.0	7.1	9.5	10.0	6.0	11.0	26.0
		HNO_3/H_2O_2	9.4	9.2	2.4	4.3	9.5	8.8	22.1	6.2	2.6	10.1	1.3	9.5

		Date	11-Feb	13-Feb	15-Feb	17-Feb	18-Feb	27-Feb	3-Mar	7-Mar	10-Mar	19-Mar	24-Mar	31-Mar
		Days	0	2	4	6	7	16	20	24	27	36	41	48
		Depth (cm)	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x	3-x
Pb	**Mean**	**NH_4Cl**	**9.88**	**5.40**	**3.74**	**3.85**	**7.49**	**6.95**	**14.59**	**12.26**	**3.78**	**16.95**	**16.79**	**6.86**
		NaOAc	**105.0**	**207.0**	**198.1**	**156.3**	**149.1**	**151.9**	**177.3**	**102.8**	**198.6**	**124.9**	**333.3**	**330.1**
		Dithionite	**105.0**	**117.3**	**130.9**	**111.9**	**101.0**	**129.1**	**121.7**	**113.1**	**117.8**	**130.9**	**140.6**	**96.0**
		HNO_3/H_2O_2	**536.8**	**623.6**	**611.5**	**705.5**	**710.5**	**577.6**	**796.2**	**1045.8**	**909.8**	**902.8**	**721.2**	**815.7**
	RSD	NH_4Cl	22.2	60.2	28.5	91.2	93.9	77.6	87.7	66.5	15.1	51.8	55.4	64.9
		NaOAc	18.3	4.5	16.1	30.4	43.7	56.0	59.7	67.4	64.7	25.9	45.6	50.3
		Dithionite	0.0	8.2	7.9	12.7	10.0	11.5	11.3	19.2	21.7	18.9	6.4	45.7
		HNO_3/H_2O_2	6.4	25.2	15.3	6.9	24.5	4.7	21.7	4.9	3.4	14.7	29.6	4.0
Zn	**Mean**	**NH_4Cl**	**68.1**	**42.5**	**34.1**	**56.5**	**140.3**	**135.3**	**216.7**	**94.2**	**120.6**	**50.6**	**622.8**	**427.4**
		NaOAc	**90.1**	**167.1**	**159.7**	**159.3**	**138.7**	**146.5**	**169.1**	**140.3**	**213.5**	**135.9**	**304.9**	**355.3**
		Dithionite	**211.0**	**208.2**	**205.7**	**301.6**	**218.9**	**253.8**	**230.3**	**211.1**	**241.3**	**275.1**	**229.1**	**213.8**
		HNO_3/H_2O_2	**1720**	**1669**	**1832**	**1998**	**1803**	**1871**	**2063**	**2720**	**2340**	**2402**	**1398**	**1270**
	RSD	NH_4Cl	19.8	33.9	53.5	99.4	73.4	92.3	69.1	106.3	152.9	42.0	80.3	130.3
		NaOAc	8.0	14.7	21.2	47.7	59.5	59.2	64.2	87.6	90.3	48.8	67.7	58.6
		Dithionite	0.0	10.5	21.0	46.1	7.9	8.5	13.6	30.4	24.4	12.4	27.0	31.4
		HNO_3/H_2O_2	10.0	11.1	13.8	9.7	6.8	11.1	11.3	32.2	16.9	15.4	84.6	61.5

APPENDIX 3

The General Linear Model derived p-values and F statistics for date and depth during the sequential extraction (Chapter 3). Degrees of freedom of date = 11, df of depth = 2.

Metal	**Factor**	**Extractant**							
		NH_4Cl		**NaOAc**		**Dithionite**		**Acid**	
		p	**F**	**p**	**F**	**p**	**F**	**p**	**F**
Cd	**Date**	0.005	2.65	0.002	2.99	< 0.001	10.58	< 0.001	6.32
	Depth	< 0.001	10.42	0.283	1.28	0.001	7.33	0.039	3.33
Cr	**Date**	< 0.001	7.77	< 0.001	27.59	< 0.001	33.15	< 0.001	12.12
	Depth	0.005	5.59	0.510	0.68	0.270	1.32	0.479	0.74
Cu	**Date**	0.005	1.44	< 0.001	4.69	< 0.001	4.83	< 0.001	6.74
	Depth	0.342	1.08	0.040	3.32	0.097	2.38	0.573	0.56
Fe	**Date**	< 0.001	10.04	0.006	3.51	< 0.001	3.90	< 0.001	14.19
	Depth	< 0.001	13.38	0.001	7.97	< 0.001	13.63	0.399	0.93
Pb	**Date**	< 0.001	3.37	0.076	1.43	< 0.001	3.40	< 0.001	7.09
	Depth	< 0.001	18.94	0.069	2.73	0.480	0.74	0.566	0.57
Zn	**Date**	< 0.001	11.87	< 0.001	7.98	0.004	2.73	0.006	2.58
	Depth	< 0.001	44.46	0.193	1.67	0.025	3.82	< 0.001	10.96

APPENDIX 4

ANOVA on pore water data (Chapter 6) showing significant differences between treatments on pore water heavy metal concentrations.

		Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	45936.584	7	6562.369	278.451	.000
	Within Groups	1484.745	63	23.567		
	Total	47421.329	70			
Zn	Between Groups	22255479.059	7	3179354.151	125.103	.000
	Within Groups	1601080.330	63	25413.973		
	Total	23856559.389	70			
Cr	Between Groups	47.167	7	6.738	205.287	.000
	Within Groups	2.068	63	.033		
	Total	49.235	70			
Pb	Between Groups	180.132	7	25.733	268.857	.000
	Within Groups	6.030	63	.096		
	Total	186.161	70			
Cd	Between Groups	289.657	7	41.380	129.480	.000
	Within Groups	20.134	63	.320		
	Total	309.791	70			

Below:

Heavy metal concentrations in pore waters pre and post growth. Values are mean (SD). Below that are the results of an ANOVA on the data and a Tamhamne posthoc test to show significiant differences between the treatments.

Pre growth

sample	Cd	Cr	Cu	Ni	Pb	Zn
No Amendment	2.872	0.436	8.252	8.22	1.439	1127.6
1% lime	0.803	0.123	1.063	2.729	1.016	383.8
2.5 % lime	0.025	0.016	0.108	0.107	0.03	6.472
5% lime	0.118	0.020	0.389	0.395	0.065	37.502
1% red mud	2.040	0.273	4.17	6.425	1.83	791.2
2.5 % red mud	1.833	0.307	4.229	6.184	2.386	776
5% red mud	1.295	0.209	2.975	4.363	1.431	599.45
1% K_2HPO_4	0.024	0.022	0.161	0.138	0.03	4.508
2.5% K_2HPO_4	0.009	0.068	0.935	0.112	0.031	0.68
5% K_2HPO_4	0.004	0.024	0.352	0.221	0.02	1.263

Post growth

sample	Cd		Cr		Cu		Ni		Pb		Zn	
No Amendment	4.64	(0.66)	2.23	(0.33)	66.08	(7.95)	12.1	(2.11)	3.87	(0.48)	1241.37	(225.91)
1% lime	2.23	(0.12)	0.15	(0.01)	7.46	(0.7)	6.28	(0.77)	3.17	(0.27)	683.87	(88.32)
2.5 % lime	0.06		0.02		0.12		0.21		0.03		23.21	
5% lime	0.00		0.01		0.14		0.02		0.02		0.33	
1% red mud	5.64	(1.06)	1.62	(0.36)	58.31	(9.66)	13.91	(3.04)	5.1	(0.3)	1487.17	(247.11)
2.5 % red mud	3.82	(0.74)	0.66	(0.12)	32.44	(4.88)	9.74	(2.07)	3.56	(0.27)	1086.36	(251.73)
5% red mud	2.80	(0.49)	0.19	(0.00)	9.72	(2.18)	6.86	(1.15)	2.84	(0.48)	766.93	(129.88)
1% K_2HPO_4	0	(0.00)	0.01	(0.00)	0.14	(0.05)	0.04	(0.004)	0.01	(0.002)	1.42	(0.42)
2.5% K_2HPO_4	0.00	(0.00)	0.03	(0.01)	0.54	(0.10)	0.12	(0.03)	0.01	(0.00)	0.46	(0.27)
5% K_2HPO_4	0.00	(0.00)	0.02	(0.00)	0.25	(0.03)	0.06	(0.01)	0.02	(0.00)	0.72	(0.14)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Cu	Between Groups	45936.584	7	6562.369	278.451	.000
	Within Groups	1484.745	63	23.567		
	Total	47421.329	70			
Zn	Between Groups	22255479.059	7	3179354.151	125.103	.000
	Within Groups	1601080.330	63	25413.973		
	Total	23856559.389	70			
Cr	Between Groups	47.167	7	6.738	205.287	.000
	Within Groups	2.068	63	.033		
	Total	49.235	70			
Pb	Between Groups	180.132	7	25.733	268.857	.000
	Within Groups	6.030	63	.096		
	Total	186.161	70			
Cd	Between Groups	289.657	7	41.380	129.480	.000
	Within Groups	20.134	63	.320		
	Total	309.791	70			

Multiple Comparisons

Tamhane

Dependent Variable	(I) Amendment	(J) Amendment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cu	1	2	58.62111(*)	2.66115	.000	46.5448	70.6975
		5	7.77111	4.17092	.908	-7.8892	23.4314
		6	33.63778(*)	3.10925	.000	21.5746	45.7010
		7	56.36000(*)	2.74856	.000	44.4775	68.2425
		8	65.99778(*)	2.65086	.000	53.8887	78.1068
		9	65.69472(*)	2.65100	.000	53.5861	77.8033
		10	65.89444(*)	2.65083	.000	53.7853	78.0036
	2	1	-58.62111(*)	2.66115	.000	-70.6975	-46.5448
		5	-50.85000(*)	3.22875	.000	-65.5331	-36.1669
		6	-24.98333(*)	1.64186	.000	-32.3563	-17.6104
		7	-2.26111	.76344	.341	-5.5059	.9837
		8	7.37667(*)	.23516	.000	6.3076	8.4457
		9	7.07361(*)	.23671	.000	6.0091	8.1381
		10	7.27333(*)	.23488	.000	6.2034	8.3432
	5	1	-7.77111	4.17092	.908	-23.4314	7.8892
		2	50.85000(*)	3.22875	.000	36.1669	65.5331
		6	25.86667(*)	3.60702	.000	11.4589	40.2744
		7	48.58889(*)	3.30117	.000	34.0892	63.0886
		8	58.22667(*)	3.22027	.000	43.5164	72.9370
		9	57.92361(*)	3.22039	.000	43.2137	72.6335
		10	58.12333(*)	3.22025	.000	43.4130	72.8337
	6	1	-33.63778(*)	3.10925	.000	-45.7010	-21.5746
		2	24.98333(*)	1.64186	.000	17.6104	32.3563
		5	-25.86667(*)	3.60702	.000	-40.2744	-11.4589
		7	22.72222(*)	1.78005	.000	15.4799	29.9646
		8	32.36000(*)	1.62513	.000	24.9369	39.7831
		9	32.05694(*)	1.62535	.000	24.6346	39.4793
		10	32.25667(*)	1.62509	.000	24.8335	39.6799
	7	1	-56.36000(*)	2.74856	.000	-68.2425	-44.4775
		2	2.26111	.76344	.341	-.9837	5.5059
		5	-48.58889(*)	3.30117	.000	-63.0886	-34.0892
		6	-22.72222(*)	1.78005	.000	-29.9646	-15.4799
		8	9.63778(*)	.72675	.000	6.3196	12.9560
		9	9.33472(*)	.72726	.000	6.0182	12.6513
		10	9.53444(*)	.72666	.000	6.2159	12.8530
	8	1	-65.99778(*)	2.65086	.000	-78.1068	-53.8887
		2	-7.37667(*)	.23516	.000	-8.4457	-6.3076
		5	-58.22667(*)	3.22027	.000	-72.9370	-43.5164
		6	-32.36000(*)	1.62513	.000	-39.7831	-24.9369
		7	-9.63778(*)	.72675	.000	-12.9560	-6.3196
		9	-.30306(*)	.03692	.000	-.4537	-.1524
		10	-.10333(*)	.02235	.009	-.1877	-.0189
	9	1	-65.69472(*)	2.65100	.000	-77.8033	-53.5861
		2	-7.07361(*)	.23671	.000	-8.1381	-6.0091

		5	-57.92361(*)	3.22039	.000	-72.6335	-43.2137
		6	-32.05694(*)	1.62535	.000	-39.4793	-24.6346
		7	-9.33472(*)	.72726	.000	-12.6513	-6.0182
		8	.30306(*)	.03692	.000	.1524	.4537
		10	.19972(*)	.03514	.007	.0492	.3502
	10	1	-65.89444(*)	2.65083	.000	-78.0036	-53.7853
		2	-7.27333(*)	.23488	.000	-8.3432	-6.2034
		5	-58.12333(*)	3.22025	.000	-72.8337	-43.4130
		6	-32.25667(*)	1.62509	.000	-39.6799	-24.8335
		7	-9.53444(*)	.72666	.000	-12.8530	-6.2159
		8	.10333(*)	.02235	.009	.0189	.1877
		9	-.19972(*)	.03514	.007	-.3502	-.0492
Zn	1	2	557.49667(*)	80.85170	.001	222.1108	892.8825
		5	-245.79778	111.60245	.706	-662.5074	170.9119
		6	155.00778	112.74516	.997	-266.2607	576.2762
		7	474.44111(*)	86.85938	.003	134.2934	814.5888
		8	1240.60222(*)	75.30252	.000	896.6157	1584.5888
		9	1241.36889(*)	75.30168	.000	897.3796	1585.3582
		10	1241.30444(*)	75.30169	.000	897.3152	1585.2937
	2	1	-557.49667(*)	80.85170	.001	-892.8825	-222.1108
		5	-803.29444(*)	87.47239	.000	-1170.5002	-436.0887
		6	-402.48889(*)	88.92571	.031	-776.6732	-28.3046
		7	-83.05556	52.35326	.983	-283.3893	117.2782
		8	683.10556(*)	29.44116	.000	548.6309	817.5802
		9	683.87222(*)	29.43900	.000	549.3905	818.3540
		10	683.80778(*)	29.43902	.000	549.3261	818.2895
	5	1	245.79778	111.60245	.706	-170.9119	662.5074
		2	803.29444(*)	87.47239	.000	436.0887	1170.5002
		6	400.80556	117.58355	.096	-37.5938	839.2049
		7	720.23889(*)	93.05360	.000	350.8599	1089.6178
		8	1486.40000(*)	82.37045	.000	1110.1255	1862.6745
		9	1487.16667(*)	82.36968	.000	1110.8897	1863.4437
		10	1487.10222(*)	82.36969	.000	1110.8252	1863.3792
	6	1	-155.00778	112.74516	.997	-576.2762	266.2607
		2	402.48889(*)	88.92571	.031	28.3046	776.6732
		5	-400.80556	117.58355	.096	-839.2049	37.5938
		7	319.43333	94.42106	.142	-56.4404	695.3071
		8	1085.59444(*)	83.91218	.000	702.2770	1468.9119
		9	1086.36111(*)	83.91143	.000	703.0412	1469.6810
		10	1086.29667(*)	83.91143	.000	702.9768	1469.6166
	7	1	-474.44111(*)	86.85938	.003	-814.5888	-134.2934
		2	83.05556	52.35326	.983	-117.2782	283.3893
		5	-720.23889(*)	93.05360	.000	-1089.6178	-350.8599
		6	-319.43333	94.42106	.142	-695.3071	56.4404
		8	766.16111(*)	43.29361	.000	568.4010	963.9212
		9	766.92778(*)	43.29214	.000	569.1628	964.6927
		10	766.86333(*)	43.29215	.000	569.0984	964.6282
	8	1	-1240.60222(*)	75.30252	.000	-1584.5888	-896.6157
		2	-683.10556(*)	29.44116	.000	-817.5802	-548.6309
		5	-1486.40000(*)	82.37045	.000	-1862.6745	-1110.1255
		6	-1085.59444(*)	83.91218	.000	-1468.9119	-702.2770
		7	-766.16111(*)	43.29361	.000	-963.9212	-568.4010

		9	.76667	.35657	.842	-.8622	2.3955
		10	.70222	.35841	.917	-.9208	2.3253
	9	1	-1241.36889(*)	75.30168	.000	-1585.3582	-897.3796
		2	-683.87222(*)	29.43900	.000	-818.3540	-549.3905
		5	-1487.16667(*)	82.36968	.000	-1863.4437	-1110.8897
		6	-1086.36111(*)	83.91143	.000	-1469.6810	-703.0412
		7	-766.92778(*)	43.29214	.000	-964.6927	-569.1628
		8	-.76667	.35657	.842	-2.3955	.8622
		10	-.06444	.03633	.966	-.2304	.1015
	10	1	-1241.30444(*)	75.30169	.000	-1585.2937	-897.3152
		2	-683.80778(*)	29.43902	.000	-818.2895	-549.3261
		5	-1487.10222(*)	82.36969	.000	-1863.3792	-1110.8252
		6	-1086.29667(*)	83.91143	.000	-1469.6166	-702.9768
		7	-766.86333(*)	43.29215	.000	-964.6282	-569.0984
		8	-.70222	.35841	.917	-2.3253	.9208
		9	.06444	.03633	.966	-.1015	.2304
Cr	1	2	2.10111(*)	.11258	.000	1.6129	2.5893
		5	.61222(*)	.16108	.044	.0108	1.2136
		6	1.57333(*)	.11556	.000	1.0882	2.0585
		7	2.15111(*)	.11072	.000	1.6593	2.6429
		8	2.23444(*)	.10882	.000	1.7373	2.7316
		9	2.23444(*)	.10882	.000	1.7373	2.7316
		10	2.23111(*)	.10887	.000	1.7342	2.7281
	2	1	-2.10111(*)	.11258	.000	-2.5893	-1.6129
		5	-1.48889(*)	.12222	.000	-2.0228	-.9550
		6	-.52778(*)	.04843	.000	-.7113	-.3443
		7	.05000	.03536	.996	-.0847	.1847
		8	.13333(*)	.02887	.047	.0015	.2652
		9	.13333(*)	.02887	.047	.0015	.2652
		10	.13000	.02906	.053	-.0013	.2613
	5	1	-.61222(*)	.16108	.044	-1.2136	-.0108
		2	1.48889(*)	.12222	.000	.9550	2.0228
		6	.96111(*)	.12497	.001	.4309	1.4914
		7	1.53889(*)	.12051	.000	1.0014	2.0764
		8	1.62222(*)	.11876	.000	1.0797	2.1648
		9	1.62222(*)	.11876	.000	1.0797	2.1648
		10	1.61889(*)	.11881	.000	1.0765	2.1613
	6	1	-1.57333(*)	.11556	.000	-2.0585	-1.0882
		2	.52778(*)	.04843	.000	.3443	.7113
		5	-.96111(*)	.12497	.001	-1.4914	-.4309
		7	.57778(*)	.04392	.000	.4034	.7522
		8	.66111(*)	.03889	.000	.4835	.8388
		9	.66111(*)	.03889	.000	.4835	.8388
		10	.65778(*)	.03903	.000	.4806	.8350
	7	1	-2.15111(*)	.11072	.000	-2.6429	-1.6593
		2	-.05000	.03536	.996	-.1847	.0847
		5	-1.53889(*)	.12051	.000	-2.0764	-1.0014
		6	-.57778(*)	.04392	.000	-.7522	-.4034
		8	.08333	.02041	.094	-.0099	.1766
		9	.08333	.02041	.094	-.0099	.1766
		10	.08000	.02068	.114	-.0125	.1725
	8	1	-2.23444(*)	.10882	.000	-2.7316	-1.7373

		2	-.13333(*)	.02887	.047	-.2652	-.0015
		5	-1.62222(*)	.11876	.000	-2.1648	-1.0797
		6	-.66111(*)	.03889	.000	-.8388	-.4835
		7	-.08333	.02041	.094	-.1766	.0099
		9	.00000	.00000	.	.0000	.0000
		10	-.00333	.00333	1.000	-.0186	.0119
	9	1	-2.23444(*)	.10882	.000	-2.7316	-1.7373
		2	-.13333(*)	.02887	.047	-.2652	-.0015
		5	-1.62222(*)	.11876	.000	-2.1648	-1.0797
		6	-.66111(*)	.03889	.000	-.8388	-.4835
		7	-.08333	.02041	.094	-.1766	.0099
		8	.00000	.00000	.	.0000	.0000
		10	-.00333	.00333	1.000	-.0186	.0119
	10	1	-2.23111(*)	.10887	.000	-2.7281	-1.7342
		2	-.13000	.02906	.053	-.2613	.0013
		5	-1.61889(*)	.11881	.000	-2.1613	-1.0765
		6	-.65778(*)	.03903	.000	-.8350	-.4806
		7	-.08000	.02068	.114	-.1725	.0125
		8	.00333	.00333	1.000	-.0119	.0186
		9	.00333	.00333	1.000	-.0119	.0186
Pb	1	2	.69889	.18281	.060	-.0191	1.4169
		5	-1.22889(*)	.18877	.000	-1.9574	-.5003
		6	.31000	.18313	.967	-.4084	1.0284
		7	1.03222(*)	.22653	.009	.1877	1.8768
		8	3.36222(*)	.16022	.000	2.6379	4.0866
		9	2.81486(*)	.17486	.000	2.1038	3.5259
		10	3.35667(*)	.16401	.000	2.6411	4.0722
	2	1	-.69889	.18281	.060	-1.4169	.0191
		5	-1.92778(*)	.13543	.000	-2.4341	-1.4215
		6	-.38889	.12745	.193	-.8640	.0863
		7	.33333	.18445	.938	-.3923	1.0590
		8	2.66333(*)	.09153	.000	2.2579	3.0688
		9	2.11597(*)	.11524	.000	1.6788	2.5531
		10	2.65778(*)	.09801	.000	2.2577	3.0578
	5	1	1.22889(*)	.18877	.000	.5003	1.9574
		2	1.92778(*)	.13543	.000	1.4215	2.4341
		6	1.53889(*)	.13586	.000	1.0311	2.0466
		7	2.26111(*)	.19035	.000	1.5253	2.9970
		8	4.59111(*)	.10292	.000	4.1324	5.0498
		9	4.04375(*)	.12448	.000	3.5671	4.5204
		10	4.58556(*)	.10872	.000	4.1340	5.0371
	6	1	-.31000	.18313	.967	-1.0284	.4084
		2	.38889	.12745	.193	-.0863	.8640
		5	-1.53889(*)	.13586	.000	-2.0466	-1.0311
		7	.72222	.18476	.052	-.0039	1.4483
		8	3.05222(*)	.09216	.000	2.6439	3.4606
		9	2.50486(*)	.11574	.000	2.0656	2.9441
		10	3.04667(*)	.09860	.000	2.6438	3.4496
	7	1	-1.03222(*)	.22653	.009	-1.8768	-.1877
		2	-.33333	.18445	.938	-1.0590	.3923
		5	-2.26111(*)	.19035	.000	-2.9970	-1.5253
		6	-.72222	.18476	.052	-1.4483	.0039

		8	2.33000(*)	.16209	.000	1.5971	3.0629
		9	1.78264(*)	.17656	.000	1.0634	2.5019
		10	2.32444(*)	.16583	.000	1.6003	3.0486
	8	1	-3.36222(*)	.16022	.000	-4.0866	-2.6379
		2	-2.66333(*)	.09153	.000	-3.0688	-2.2579
		5	-4.59111(*)	.10292	.000	-5.0498	-4.1324
		6	-3.05222(*)	.09216	.000	-3.4606	-2.6439
		7	-2.33000(*)	.16209	.000	-3.0629	-1.5971
		9	-.54736(*)	.07438	.002	-.8900	-.2047
		10	-.00556	.04309	1.000	-.1806	.1695
	9	1	-2.81486(*)	.17486	.000	-3.5259	-2.1038
		2	-2.11597(*)	.11524	.000	-2.5531	-1.6788
		5	-4.04375(*)	.12448	.000	-4.5204	-3.5671
		6	-2.50486(*)	.11574	.000	-2.9441	-2.0656
		7	-1.78264(*)	.17656	.000	-2.5019	-1.0634
		8	.54736(*)	.07438	.002	.2047	.8900
		10	.54181(*)	.08222	.001	.2059	.8778
	10	1	-3.35667(*)	.16401	.000	-4.0722	-2.6411
		2	-2.65778(*)	.09801	.000	-3.0578	-2.2577
		5	-4.58556(*)	.10872	.000	-5.0371	-4.1340
		6	-3.04667(*)	.09860	.000	-3.4496	-2.6438
		7	-2.32444(*)	.16583	.000	-3.0486	-1.6003
		8	.00556	.04309	1.000	-.1695	.1806
		9	-.54181(*)	.08222	.001	-.8778	-.2059
Cd	1	2	2.42000(*)	.25005	.000	1.4328	3.4072
		5	-.99667	.41723	.601	-2.6132	.6199
		6	.82556	.33097	.495	-.4117	2.0628
		7	1.84778(*)	.27288	.000	.8133	2.8823
		8	4.54111(*)	.22047	.000	3.5409	5.5413
		9	4.39153(*)	.22588	.000	3.4041	5.3789
		10	4.50778(*)	.22204	.000	3.5120	5.5035
	2	1	-2.42000(*)	.25005	.000	-3.4072	-1.4328
		5	-3.41667(*)	.37440	.000	-4.9998	-1.8336
		6	-1.59444(*)	.27503	.003	-2.7004	-.4885
		7	-.57222	.20140	.298	-1.3357	.1912
		8	2.12111(*)	.12128	.000	1.5793	2.6629
		9	1.97153(*)	.13084	.000	1.4376	2.5055
		10	2.08778(*)	.12411	.000	1.5516	2.6240
	5	1	.99667	.41723	.601	-.6199	2.6132
		2	3.41667(*)	.37440	.000	1.8336	4.9998
		6	1.82222(*)	.43266	.023	.1719	3.4726
		7	2.84444(*)	.39002	.000	1.2627	4.4262
		8	5.53778(*)	.35533	.000	3.9189	7.1566
		9	5.38819(*)	.35871	.000	3.7792	6.9972
		10	5.50444(*)	.35630	.000	3.8886	7.1203
	6	1	-.82556	.33097	.495	-2.0628	.4117
		2	1.59444(*)	.27503	.003	.4885	2.7004
		5	-1.82222(*)	.43266	.023	-3.4726	-.1719
		7	1.02222	.29593	.105	-.1158	2.1602
		8	3.71556(*)	.24844	.000	2.5868	4.8443
		9	3.56597(*)	.25325	.000	2.4495	4.6824
		10	3.68222(*)	.24983	.000	2.5576	4.8068

7	1	-1.84778(*)	.27288	.000	-2.8823	-.8133
	2	.57222	.20140	.298	-.1912	1.3357
	5	-2.84444(*)	.39002	.000	-4.4262	-1.2627
	6	-1.02222	.29593	.105	-2.1602	.1158
	8	2.69333(*)	.16323	.000	1.9569	3.4297
	9	2.54375(*)	.17046	.000	1.8199	3.2676
	10	2.66000(*)	.16534	.000	1.9289	3.3911
8	1	-4.54111(*)	.22047	.000	-5.5413	-3.5409
	2	-2.12111(*)	.12128	.000	-2.6629	-1.5793
	5	-5.53778(*)	.35533	.000	-7.1566	-3.9189
	6	-3.71556(*)	.24844	.000	-4.8443	-2.5868
	7	-2.69333(*)	.16323	.000	-3.4297	-1.9569
	9	-.14958	.05658	.534	-.3966	.0975
	10	-.03333	.03851	1.000	-.1832	.1165
9	1	-4.39153(*)	.22588	.000	-5.3789	-3.4041
	2	-1.97153(*)	.13084	.000	-2.5055	-1.4376
	5	-5.38819(*)	.35871	.000	-6.9972	-3.7792
	6	-3.56597(*)	.25325	.000	-4.6824	-2.4495
	7	-2.54375(*)	.17046	.000	-3.2676	-1.8199
	8	.14958	.05658	.534	-.0975	.3966
	10	.11625	.06242	.923	-.1326	.3651
10	1	-4.50778(*)	.22204	.000	-5.5035	-3.5120
	2	-2.08778(*)	.12411	.000	-2.6240	-1.5516
	5	-5.50444(*)	.35630	.000	-7.1203	-3.8886
	6	-3.68222(*)	.24983	.000	-4.8068	-2.5576
	7	-2.66000(*)	.16534	.000	-3.3911	-1.9289
	8	.03333	.03851	1.000	-.1165	.1832
	9	-.11625	.06242	.923	-.3651	.1326

* The mean difference is significant at the .05 level.

APPENDIX 5

Observations of *Lolium multiflorum* appearance and height during the growth period (Chapter 6). *Lolium* was planted on 22nd August.

10th September 2003 (20 d after planting)

			Growth							
Pot	rep	pH	very poor	poor	poor-med	med	med-good	good	Max height (cm)	Observations
No Amendment	1		/////////							Green to red to orange at the tips
	2	3.55	/////////						1.5	Growth around rhizon sampler. Orange tips
	3		/////////						1	Green
1% Lime	1					/////////			5.5	Stems light green to purple to red at tips
	2	4.4				////////			5	Mid to tips = yellow to purple, purple hard and sturdy, yellow floppy.
	3				////////				5	Light green to yellow, purple/brown at tips
2.5% Lime	1							/////////	14.5	Tips yellow to brown and dead
	2	6.35						/////////	16	Tips yellow to dead
	3							/////////	15	Tips yellow to dead
5% Lime	1							/////////	15	Very slight yellowing of tips
	2	7.15						/////////	18	Slight yellowing of tips
	3							/////////	15.5	Tips orange to dead
1% Red mud	1			/////////					2	Yellow to orange at tips
	2	3.67	/////////						1.5	Light green to yellow/orange tips. Soil compacted.

10th September 2003	**3**			//////////					3	**50% tips yellow and curled**
2.5% Red mud	**1**	**3.89**		//////////					3	**50% tips yellow and curled**
	2			//////////					4	**Tips yellow to red**
	3			//////////					6	**Light green to yellow to orange tips.**
5% Red mud	**1**	**4.34**					//////////		7	**Tips yellow to red**
	2				//////////					**Green to yellow to purple. Veins darker green in light green/yellow stems**
	3					//////////			9.5	**Yellow to orange to red at tips**
1% K2HPO4, 5% Lime	**1**	**6.9**						//////////	15.5	**Tips curled, orange/yellow/dead**
	2							//////////	14	**Tips yellow/dead**
	3							//////////	19.5	**Tips yellow/dead**
2.5% K2HPO4, 5% Lime	**1**	**7.2**				//////////			12	**Soil compacted. Tips curling but not dead.**
	2							//////////	14	**80% dying/shrivelling at tips**
	3							//////////	15	**50% unhealthy, light green stems. 80% tips curling**
5% K2HPO4, 5% Lime	**1**	**6.82**						//////////	23.5	**60% tips curled**
	2							//////////	19.5	**30% tips curled but fine, not dead**
	3							//////////	18.5	**40% tips curled/dead**
5% red mud, 5% lime	**1**								3	**Grass started to grow, healthy**
	2								2.5	**1 seedling**
	3								1.5	**1 plant**

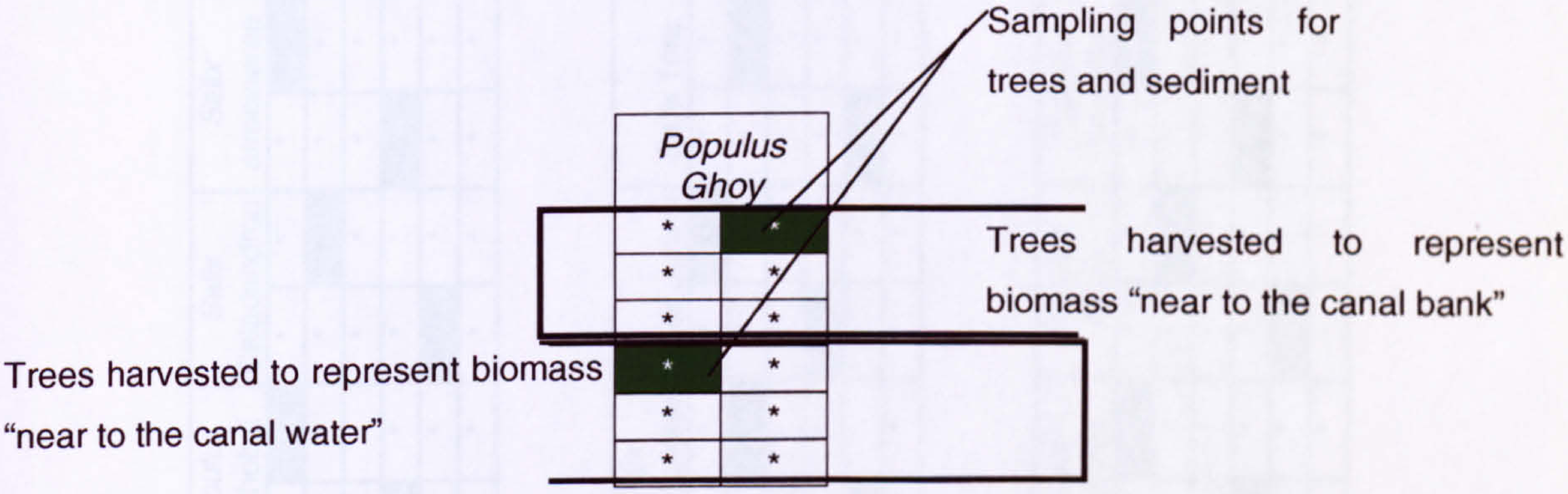
30th September 2003 (40 d after planting)

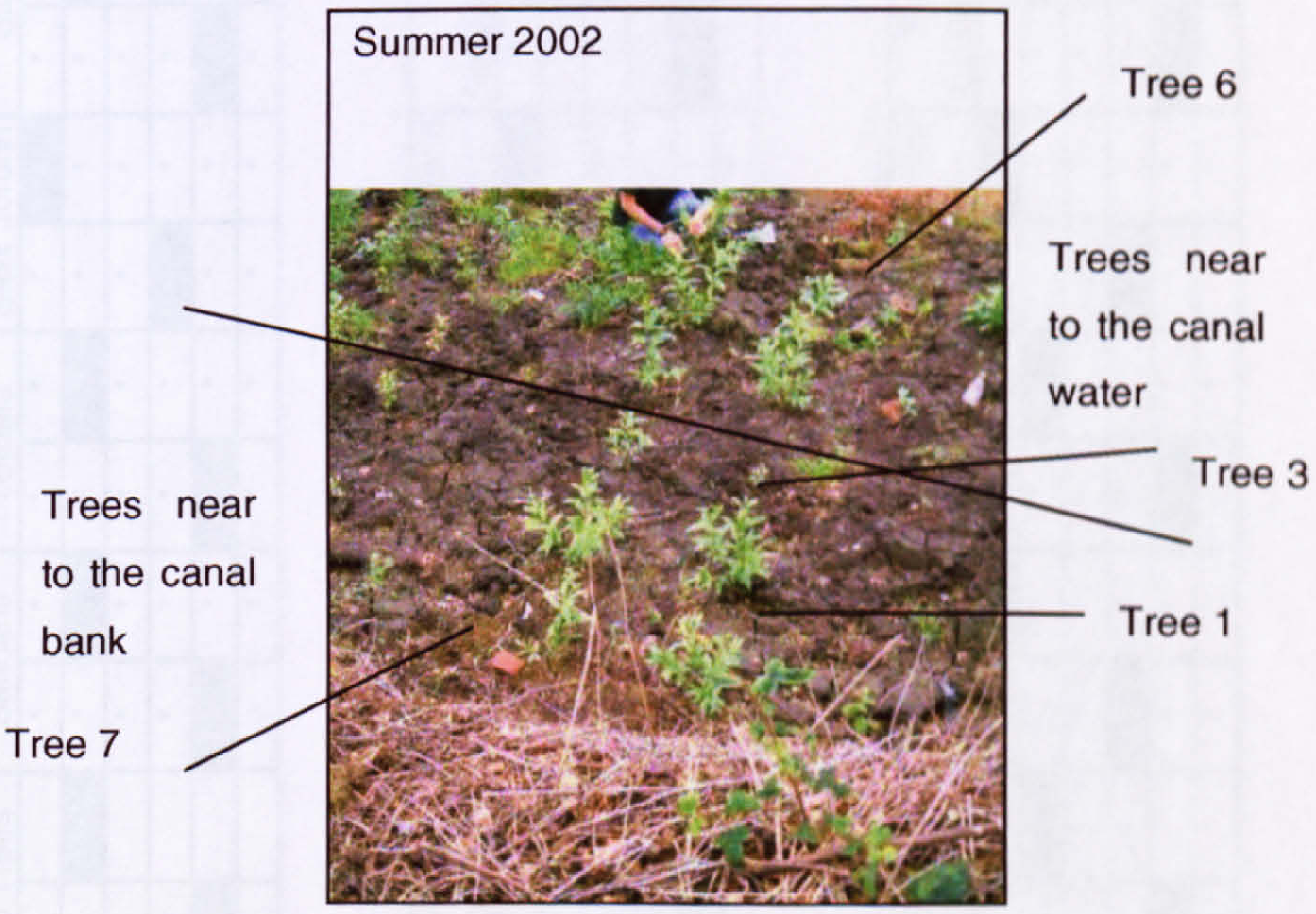
		Growth							
Pot	rep	very poor	poor	poor-med	med	med-good	good	Max height (cm)	Observations
No Amendment	1	//////////						1.5	Yellow to orange. Dead?
	2	//////////						1	Purple or orange. Dead?
	3	//////////						1	Purple/yellow/orange. Dead?
1% Lime	1				//////////			6	Yellow to purple. Tips curled.
	2			//////////				5.5	Yellow to purple. Tips curled.
	3			//////////				5.5	Yellow to purple. Tips curled.
2.5% Lime	1						//////////	19.5	Tips yellow to Purple/dead
	2						//////////	20.5	20% dead. Tips/stems green to orange/yellow
	3						//////////	18	20% dead. Half stems yellow to orange to dead
5% Lime	1						//////////	19.5	Tips yellow/brown/dead
	2						//////////	19.5	Much yellowing. Tips red to yellow and curled. 20% dead
	3						//////////	23	Stems green to yellow to orange
1% Red mud	1		//////////					1.5	Purple to brown
	2		//////////					1	Purple to yellow
	3		//////////					2.5	10% green, rest yellow to orange to purple
2.5% Red mud	1		//////////					2.5	10% green, rest yellow to purple
	2		//////////					3	light green to purple to yellow. Some curled.

(30th September 2003)	3			//////////				5.3	Green to purple to yellow to curled
5% Red mud	1				//////////			8.5	Green to purple to yellow. Some tips curled
	2				//////////			6	Green to tips purple to purple moves down stem, to tips yellow to yellow down stem. 2 purple stems
	3				//////////			7	green to purple to yellow to curled tips
1% K2HPO4	1						//////////	19.5	20% dead. Tips yellow/orange to purple. Some curled
5% Lime	2						//////////	20	20% dead. Tips long, curled/yellow to dead
	3						//////////	23.5	20% dead. Tips long, yellow/purple/curled/dead. Light green foliage
2.5% K2HPO4	1						//////////	26.5	Bushy. Tips purple to yellow. Dark green
5% Lime	2						//////////	21	Thick tall bushy stems. Small tips, purple to yellow. Dark green foliage
	3						//////////	25	Big, thick, tall stems, 10% dead, rest green to slightly purple tips. Dark green foliage
5% K2HPO4	1						//////////	28	Ends yellow/pink/purple/orange and bit curled.
5% Lime	2						//////////	28	Green. 50% pink to yellow tips to stem. 5% dead
	3						//////////	24.5	Green to purple tips to yellow to orange
5% red mud	1						//////////	29	Quite light green. 20% yellow. 90% tips yellow
5% Lime	2						//////////	22	Quite light green. 20% yellow. 60% tips yellow
	3						//////////	22	Light green but healthy. Tip ends yellow to dead. Some pink

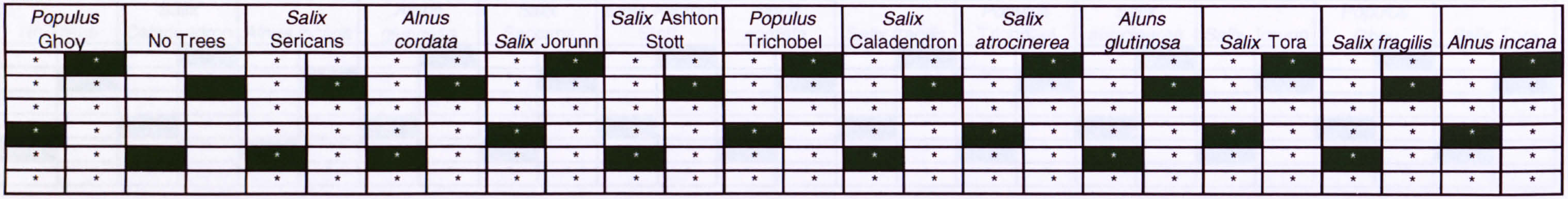
APPENDIX 6

How biomass was measured at the canal site (Chapter 7) and the canal map plan, with sampled trees/sediment marked. The bank/wall end of the plot is nearest to the species name (i.e. at the top). The bottom of the plot bordered the water





Block A



Populus Ghoy		No Trees		*Salix* Sericans		*Alnus cordata*		*Salix* Jorunn		*Salix* Ashton Stott		*Populus* Trichobel		*Salix* Caladendron		*Salix atrocinerea*		*Aluns glutinosa*		*Salix* Tora		*Salix fragilis*		*Alnus incana*	
*	■ *			*	*	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *
*	*		■	*	■ *	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*
*	*			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
■ *	*			*	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*
*	*	■		■ *	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*
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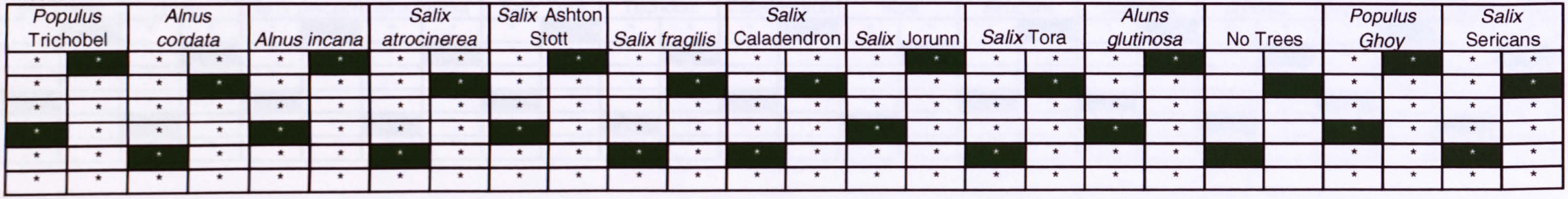
Block B



Populus Trichobel		*Alnus cordata*		*Alnus incana*		*Salix atrocinerea*		*Salix* Ashton Stott		*Salix fragilis*		*Salix* Caladendron		*Salix* Jorunn		*Salix* Tora		*Aluns glutinosa*		No Trees		*Populus Ghoy*		*Salix* Sericans	
*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	*	*	■ *	*	*	*	■ *			*	■ *	*	*
*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	■ *	*	*	*	■ *	*	*		■	*	*	*	■ *
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*	*	*
■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	*	*	■ *	*	*	*	■ *	*			■ *	*	*	*
*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	■ *	*	*	*	■ *	*	*	*	■		*	*	■ *	*
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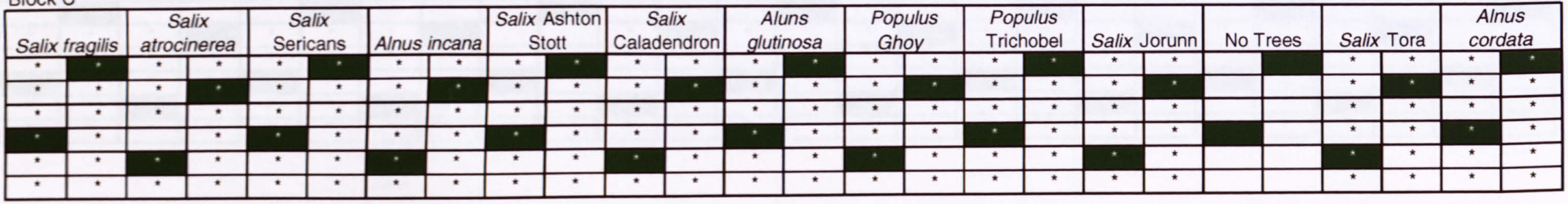
Block C



Salix fragilis		*Salix atrocinerea*		*Salix* Sericans		*Alnus incana*		*Salix* Ashton Stott		*Salix* Caladendron		*Aluns glutinosa*		*Populus Ghoy*		*Populus* Trichobel		*Salix* Jorunn		No Trees		*Salix* Tora		*Alnus cordata*	
*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*		■	*	*	*	■ *
*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *			*	■ *	*	*
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■ *	*		*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■		*	*	■ *	*
*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*	*	*	■ *	*			■ *	*	*	*
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Block D

No Trees		*Salix* Caladendron		*Alnus incana*		*Aluns glutinosa*		*Salix* Sericans		*Salix* Ashton Stott		*Alnus cordata*		*Salix fragilis*		*Populus* Trichobel		*Salix atrocinerea*		*Salix* Jorunn		*Populus Ghoy*		*Salix* Tora	
		*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*
	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■
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■		*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*
		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Block E

No Trees		*Populus Ghoy*		*Salix* Jorunn		*Salix atrocinerea*		*Salix* Tora		*Populus* Trichobel		*Salix* Caladendron		*Salix* Ashton Stott		*Salix* Sericans		*Aluns glutinosa*		*Alnus cordata*		*Alnus incana*		*Salix fragilis*	
	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	■	*	*	*	■	*	*
		*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	*	*	■	*	*	*	■
		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
■		*	*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	■	*	*	*	■	*	*	*
		■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	*	*	■	*	*	*	■	*
		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

Block F

Salix Ashton Stott		*Salix atrocinerea*		*Salix* Jorunn		*Alnus cordata*		No Trees		*Alnus incana*		*Aluns glutinosa*		*Populus* Trichobel		*Salix* Tora		*Populus Ghoy*		*Salix* Caladendron		*Salix* Sericans		*Salix fragilis*	
*	■	*	*	*	■	*	*		■	*	*	*	■	*	*	*	■	*	*	*	■	*	*	*	■
*	*	*	■	*	*	*	■			*	■	*	*	*	■	*	*	*	■	*	*	*	■	*	*
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APPENDIX 7

Observations of experimental trees during the late 2003 and 2004 growing seasons at the canal site (Chapter 7).

Appendix Key

Dead = Trees which were found to be dead at the time of the survey.

Eaten = Trees whose leaves showed some signs of herbivory, i.e. some holes in some leaves.

v. Eaten = Trees whose leaves showed signs of herbivory i.e. many holes in many leaves.

v.v. Eaten = trees whose leaves showed signs of very significant herbivory i.e. Many holes in many leaves and some leaves eaten back to the spine.

Toxicity = Trees whose leaves show mild toxicity symptoms i.e. slight chlorosis.

v. Toxicity = Trees whose leaves show worse toxicity symptoms, i.e. more severe chlorosis.

v.v. Toxicity = Trees whose leaves show even worse toxicity symptoms i.e. chlorosis in all leaves, and smaller leaf size.

v.v.v. Toxicity = Trees whose leaves show signs of severe toxicitiy i.e. severe chlorosis in all leaves, and necrosis at leaf edges, twisted leaves etc.

v.v.v.v. Toxicity = Trees whose leaves are extremely chlorotic and experiencing leaf necrosis in most leaves, with a significant percentage of the leaf being dead.

Diseased = Trees whose leaves exhibited obvious symptoms of disease e.g. mottling with red/orange spot in the case of *Populus* Trichobel. N.B. Only measured in 2004. The poplar Trichobel was diseased in 2003 also, but no survey of the percentage of trees suffering from disease was noted.

N.B. Diseased, eaten and "toxicity" trees are not mutually exclusive and a tree may exhibit any combination of these symptoms. However, different degrees of "toxicity" or "eaten" are exclusive from other degrees of the same symptom.

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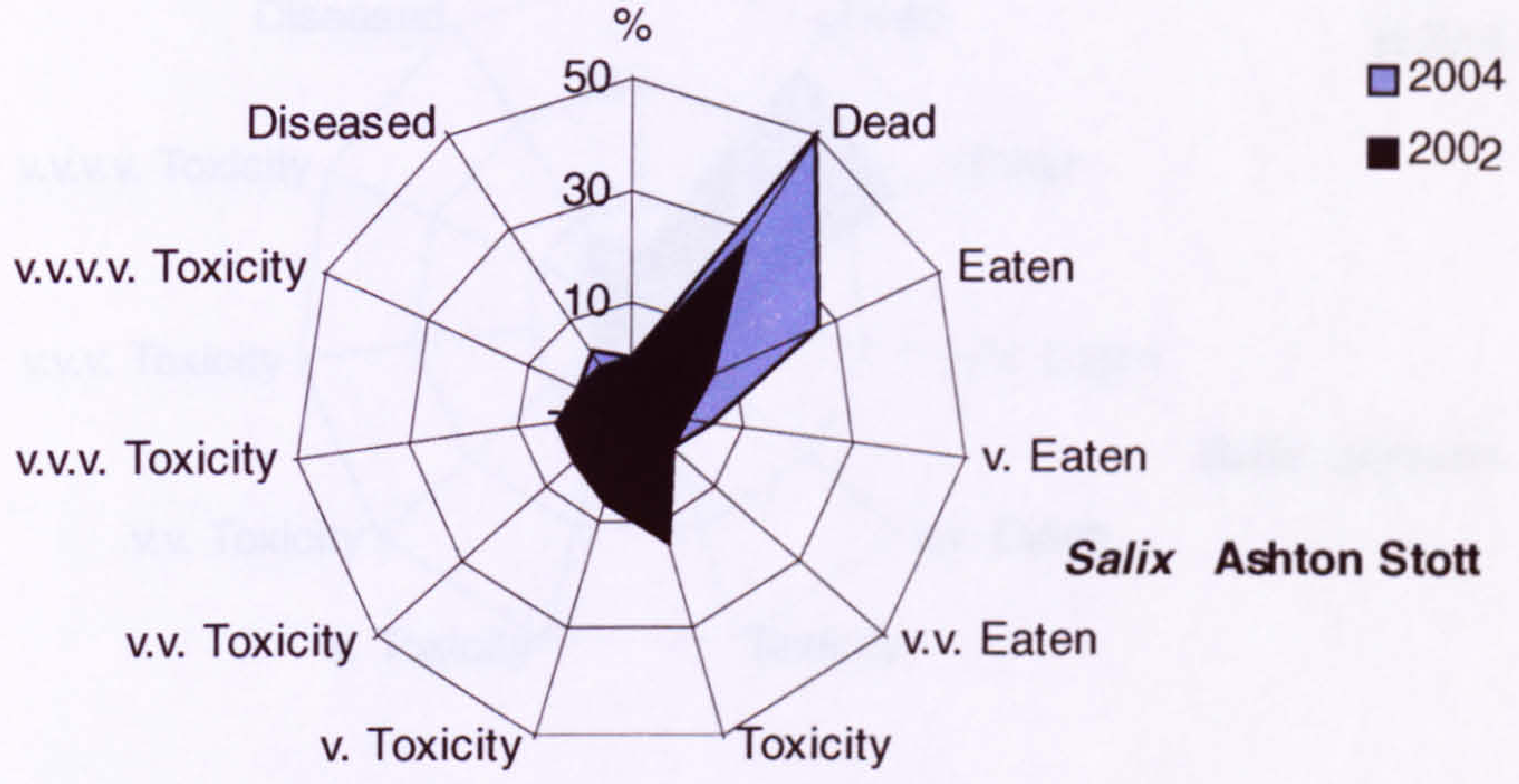
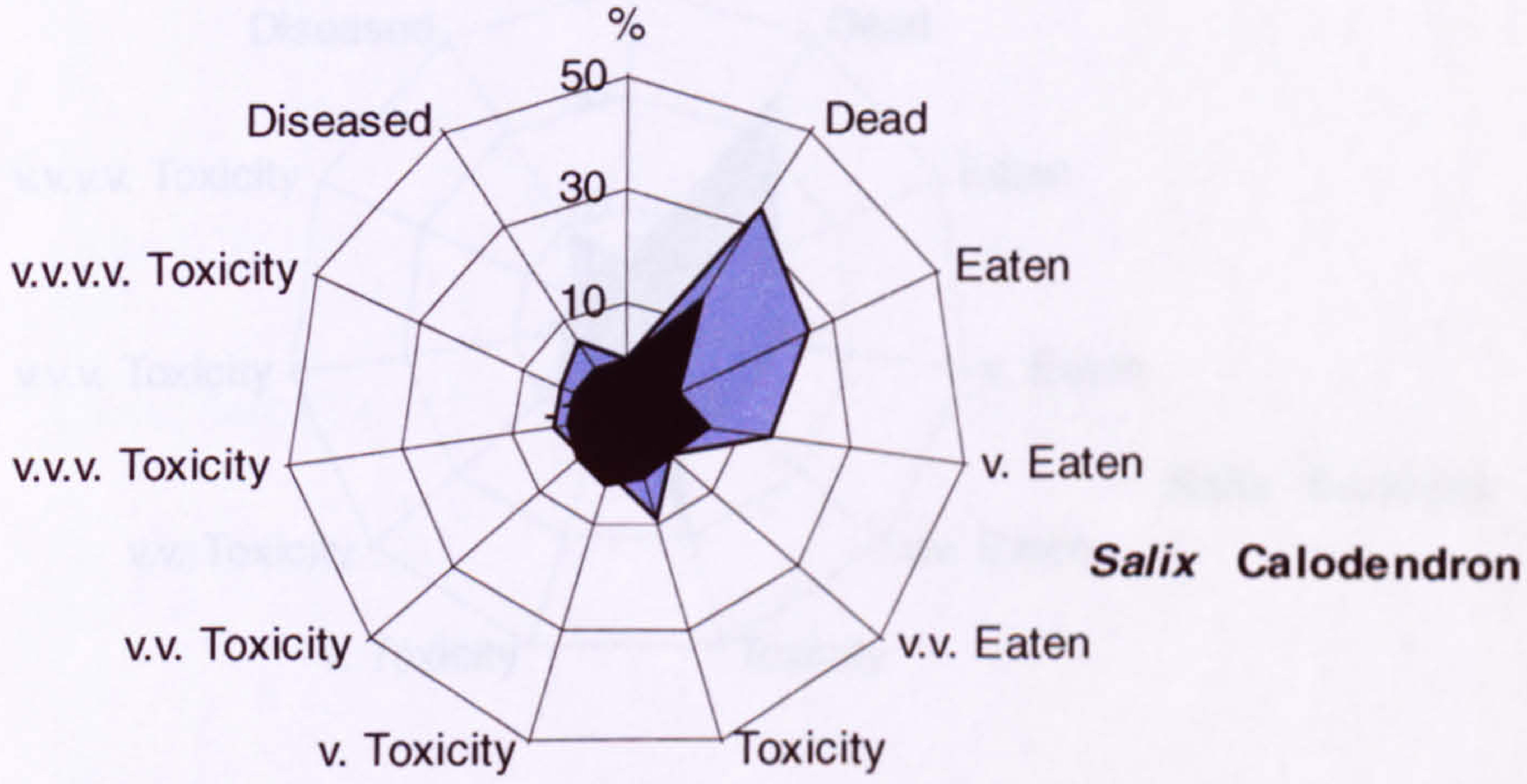


***Salix* Ashton Stott**



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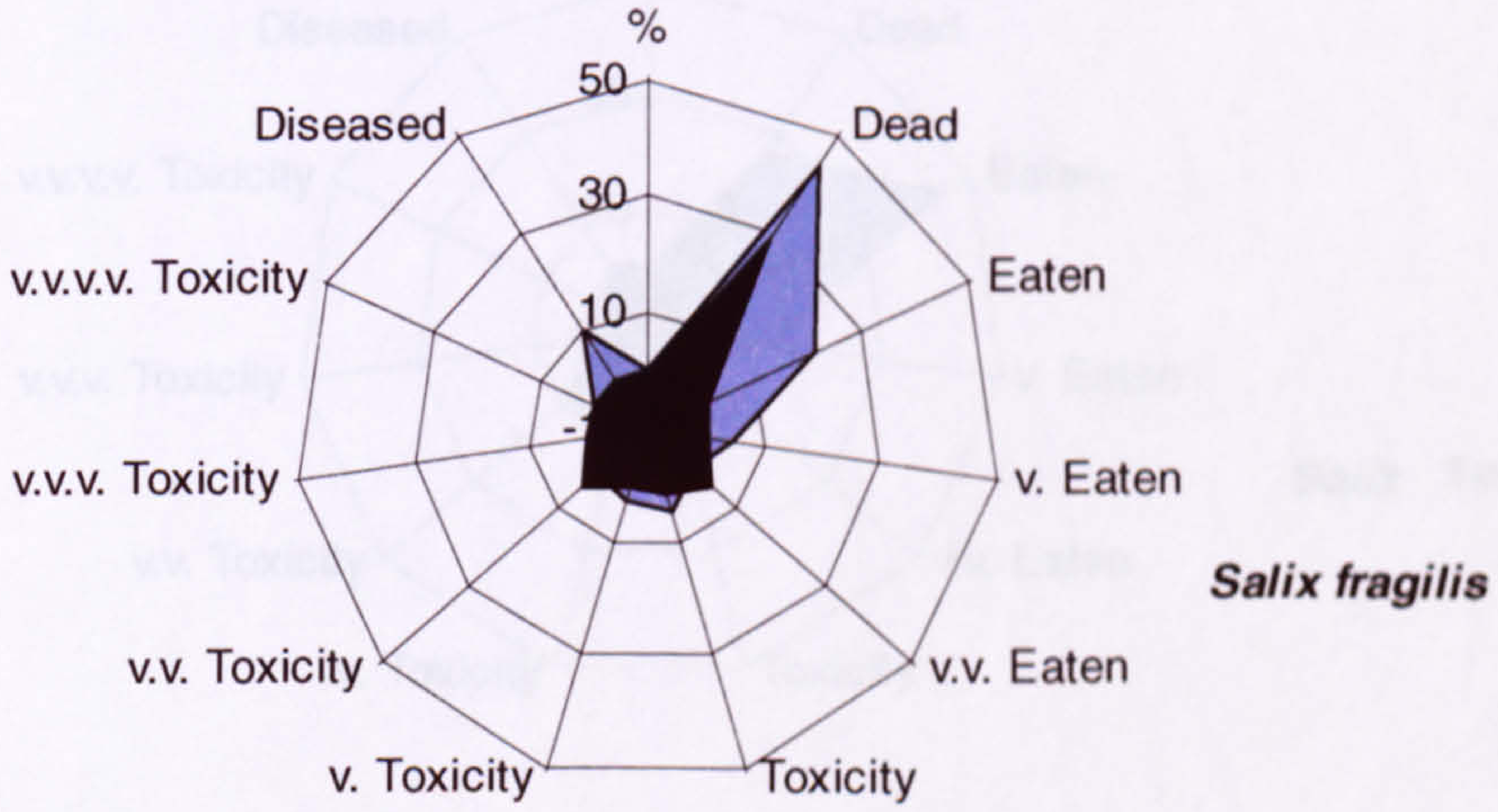
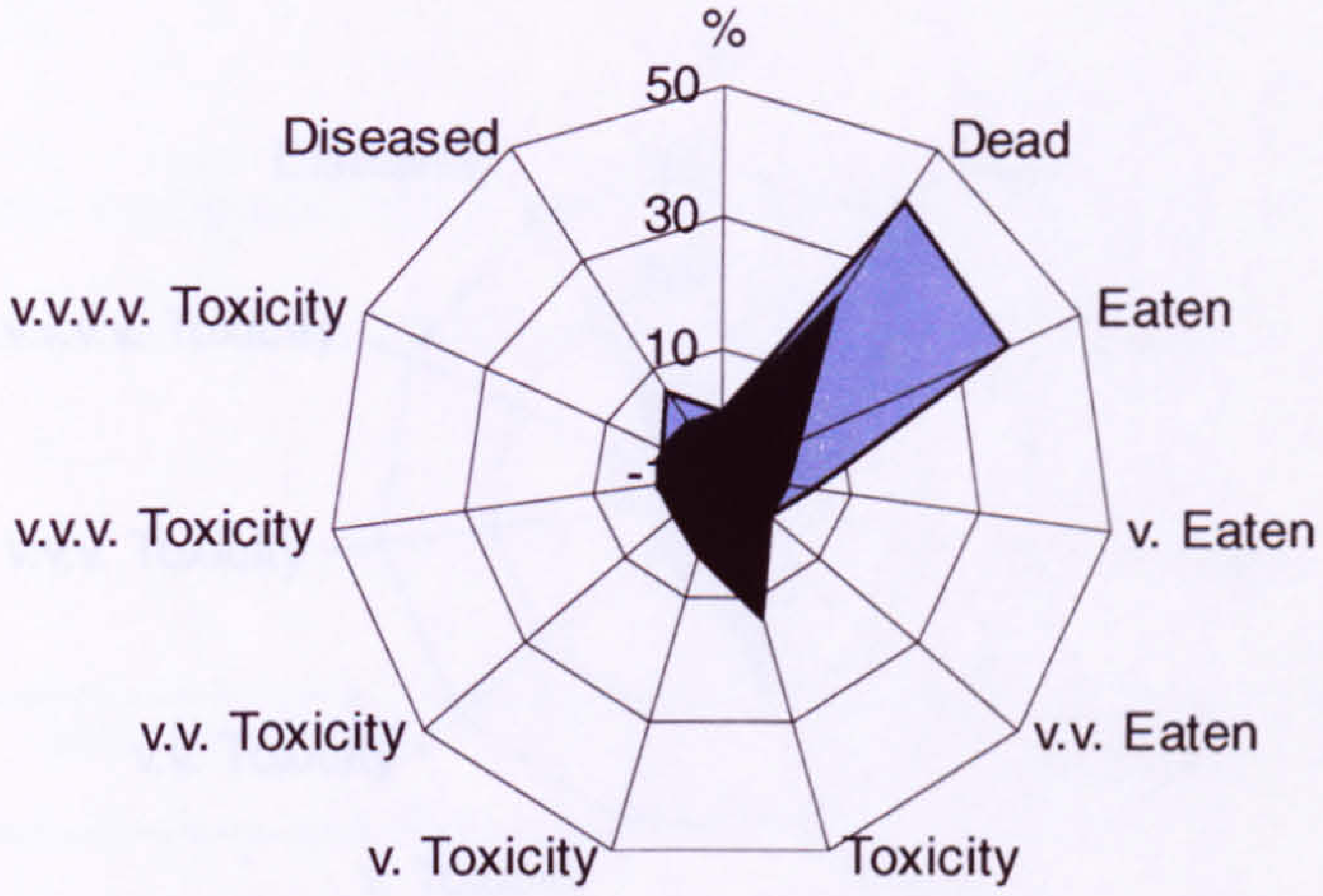
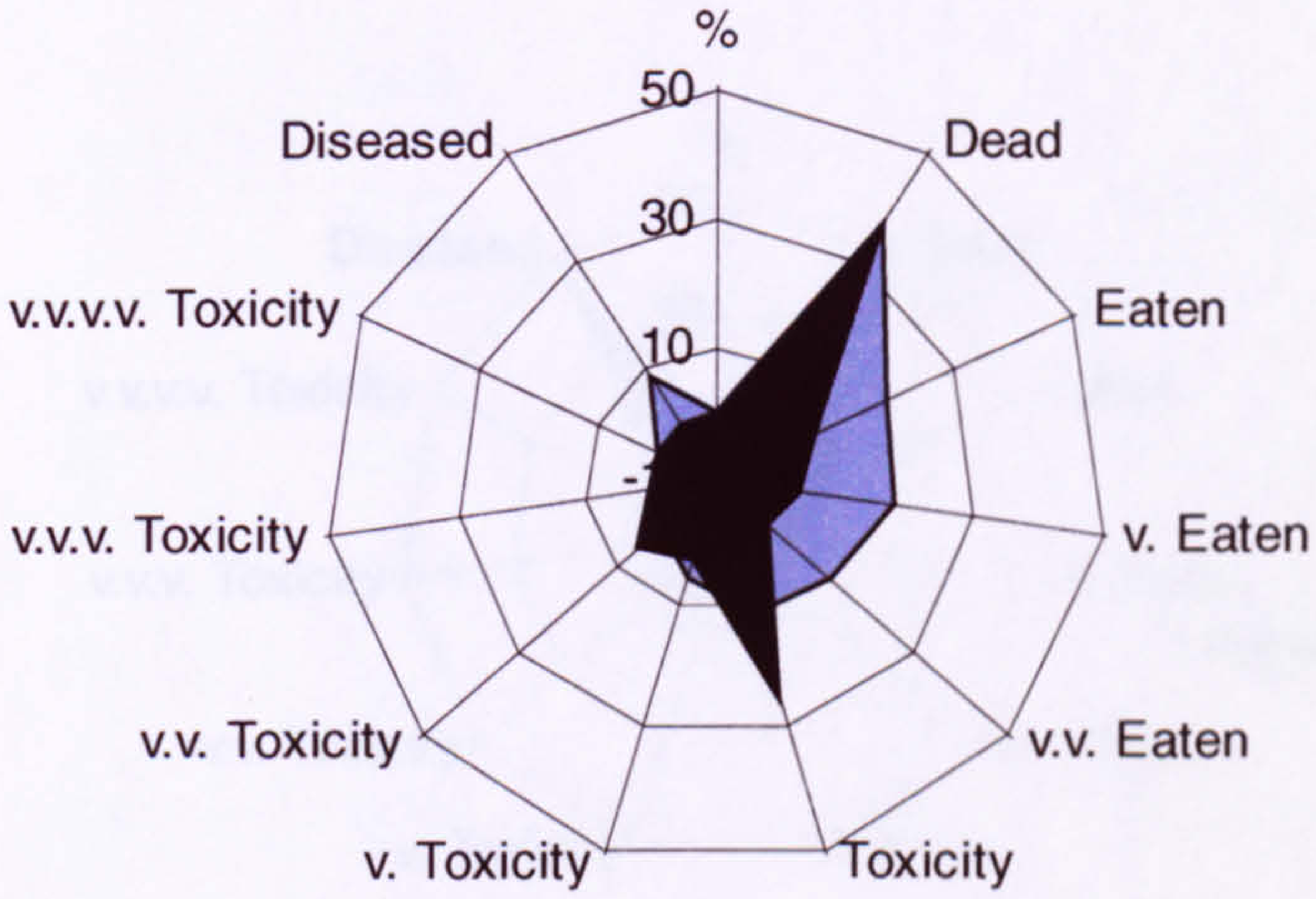


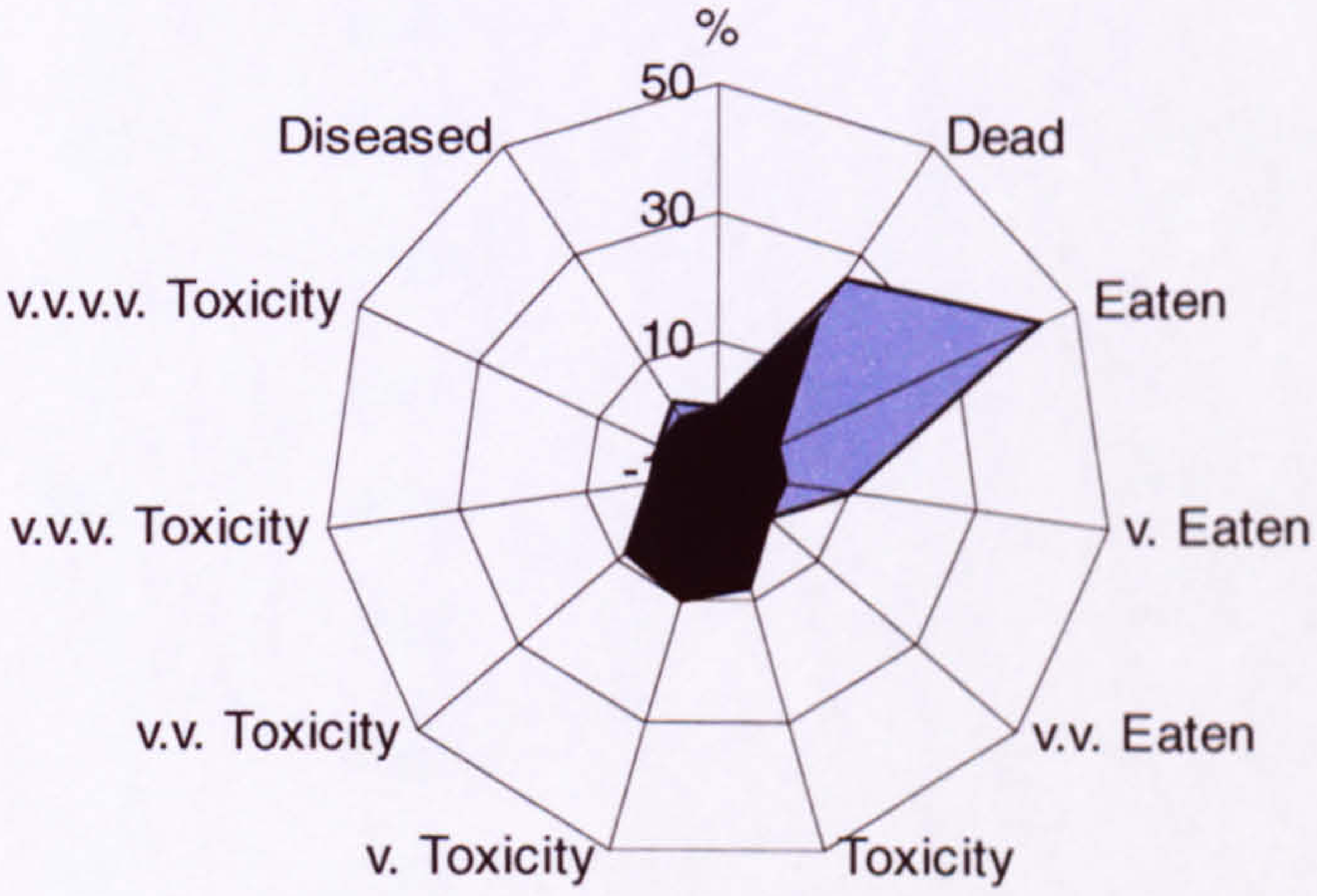
Salix fragilis



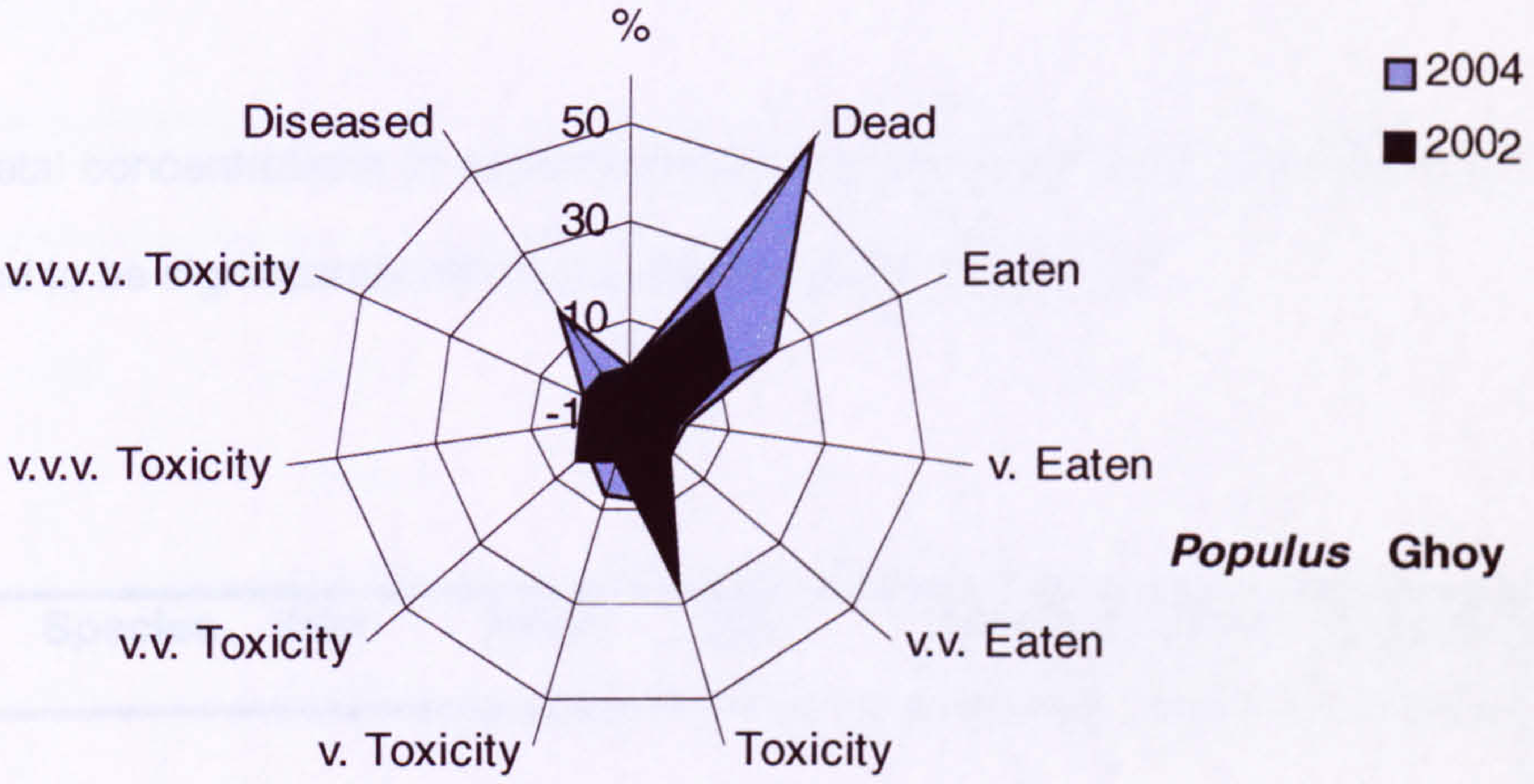
***Salix* Jorrunn**



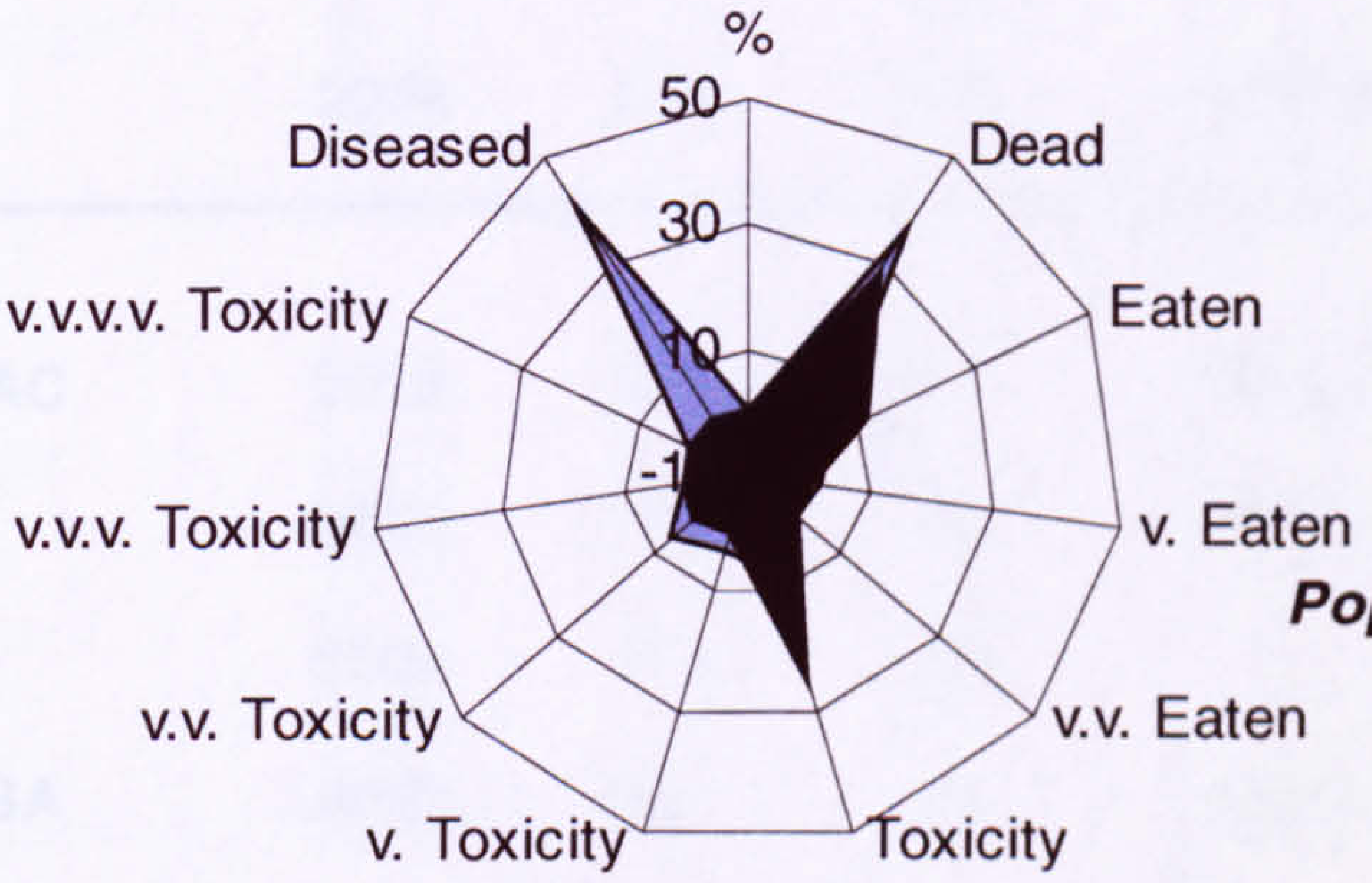
***Salix* Sericans**



***Salix* Tora**



***Populus* Ghoy**



***Populus* Trichobel**

APPENDIX 8

Heavy metal concentrations in experimental tree leaves and stems from the field site, where they were found to be significantly different between years (Chapter 7).

Leaves

Metal	Species	Year	Mean	SD	Differing Years	p-value
Cd	SAS	2002	1.67	0.58		
		2003	3.00	1.10		
		2004	1.56	0.75	2003/2004	0.032
Cu	AC	2002	10.1	4.8	2002/2003	0.017
		2003	15.6	4.6	2002/2004	0.031
		2004	14.5	4.6		
	SA	2002	10.1	2.0	2002/2003	0.042
		2003	7.9	2.0		
		2004	-	-		
	SC	2002	12.93	3.19	2002/2003	0.021
		2003	9.96	2.51		
		2004	13.2	6.3		
	SJ	2002	12.0	2.0	2002/2003	0.005
		2003	8.0	1.9		
		2004	10.8	5.7		
	ST	2002	13.2	3.0		
		2003	10.4	3.9	2002/2004	0.043
		2004	10.9	2.2		

Leaves

Metal	Species	Year	Mean	SD	Differing Years	p-value
Pb	PG	2002	8.2	5.1	2002/2003	0.027
		2003	3.1	3.3		
		2004	3.2	3.0		
	SA	2002	6.2	3.0	2002/2003	0.042
		2003	2.1	2.6		
		2004	-	-		
	SAS	2002	3.9	1.7		
		2003	2.5	1.0	2002/2004	0.018
		2004	1.8	1.4		
	SJ	2002	3.3	1.0		
		2003	4.0	2.4	2002/2004	0.015
		2004	2.0	0.6	2003/2004	0.020
Zn	PT	2002	616	190	2002/2003	0.028
		2003	1216	598		
		2004	755	173		

Stems

Metal	Species	Year	Mean	SD	p-value
Cd	SAS	2003	4.00	1.58	0.004
		2004	1.52	0.80	
	SC	2003	3.59	3.40	0.020
		2004	1.66	0.73	
	SF	2003	2.50	1.51	0.036
		2004	0.20	0.10	
Cu	PT	2003	5.1	1.1	0.010
		2004	2.5	2.7	
	SF	2003	10.7	4.1	0.036
		2004	2.7	2.0	
	SJ	2003	7.8	1.3	0.033
		2004	4.4	4.1	
Pb	SJ	2003	2.7	1.9	0.041
		2004	1.3	1.6	
Zn	ST	2003	364	80	0.043
		2004	531	139	

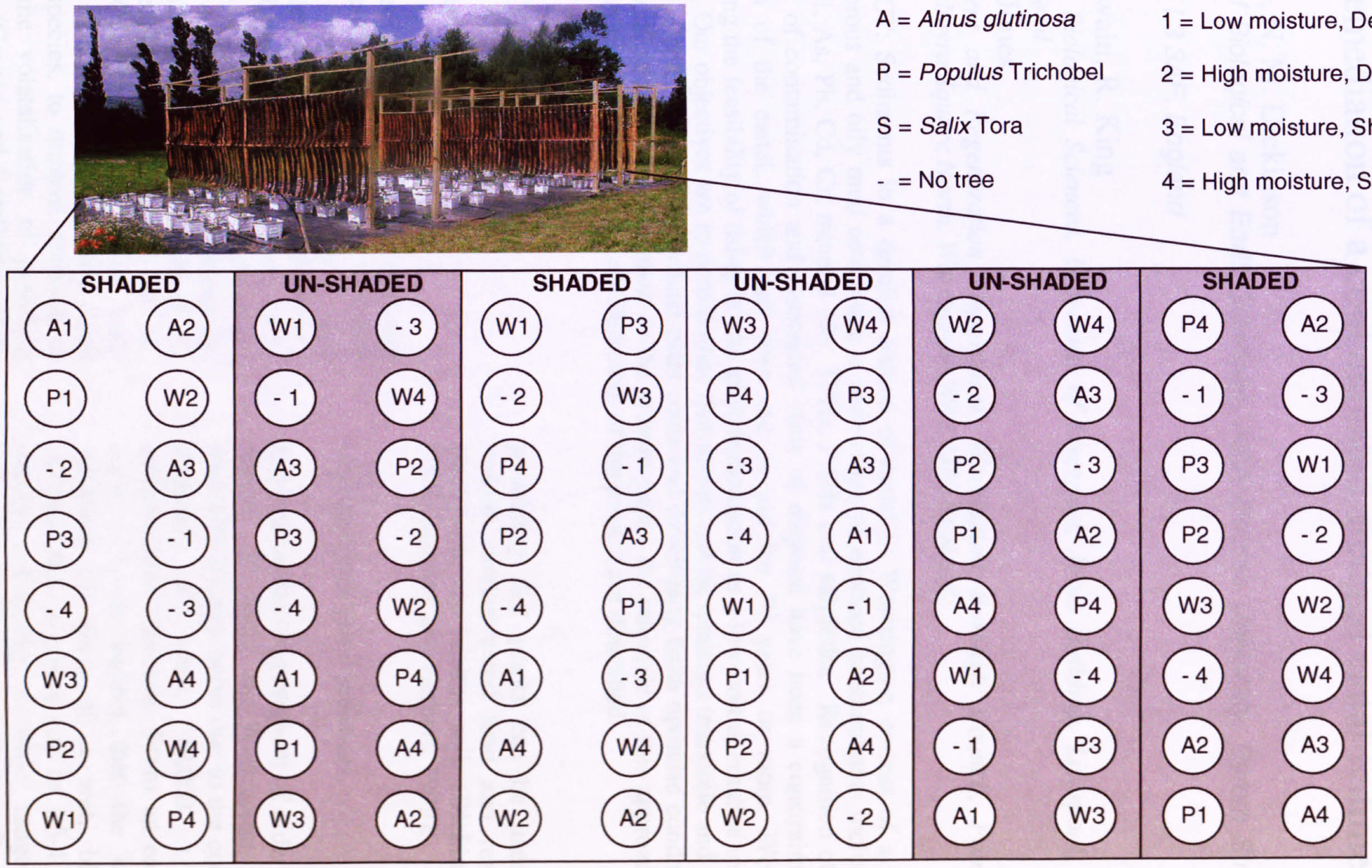
APPENDIX 9

Layout of the experiment described in Chapter 8

KEY

A = *Alnus glutinosa*
P = *Populus* Trichobel
S = *Salix* Tora
- = No tree

1 = Low moisture, Deep sediment
2 = High moisture, Deep sediment
3 = Low moisture, Shallow sediment
4 = High moisture, Shallow sediment



SHADED		UN-SHADED		SHADED		UN-SHADED		UN-SHADED		SHADED	
A1	A2	W1	- 3	W1	P3	W3	W4	W2	W4	P4	A2
P1	W2	- 1	W4	- 2	W3	P4	P3	- 2	A3	- 1	- 3
- 2	A3	A3	P2	P4	- 1	- 3	A3	P2	- 3	P3	W1
P3	- 1	P3	- 2	P2	A3	- 4	A1	P1	A2	P2	- 2
- 4	- 3	- 4	W2	- 4	P1	W1	- 1	A4	P4	W3	W2
W3	A4	A1	P4	A1	- 3	P1	A2	W1	- 4	- 4	W4
P2	W4	P1	A4	A4	W4	P2	A4	- 1	P3	A2	A3
W1	P4	W3	A2	W2	A2	W2	- 2	A1	W3	P1	A4

APPENDIX 10

Text for Royle *et al.*, (2003) with the spelling mistake "*Poplus*" corrected to "*Populus*" in Table 1

Phytoremediation of a contaminated dredged canal sediment

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ABSTRACT: Sediments in a derelict section of canal in Warrington consist of a wet, black, odorous and oily mud containing a wide range of elevated contaminants including Cu, Zn. Ni, As, Pb, Cd, Cr, mineral oils, TPHs, PAHs and sulphides. Recognition of the high level of contamination and associated costs of disposal have been a constraint for restoration of the canal, which has been out of use for 50 years or more. We are investigating the feasibility of using *in situ* phytoremediation as a low-cost alternative to cart and dump. Our objectives are to demonstrate that metals can be rendered immobile and non-hazardous in soils and biomass, whilst plant roots and developing biota optimise conditions for the natural attenuation of organics. In the present paper, the rationale of this approach to reclamation and the first year of establishment of the project are described.

INTRODUCTION

Phytoremediation

Phytoremediation is receiving considerable attention as a low-cost treatment technology for land and groundwater contaminated with heavy metals (Chaney et al., 1997, Glass, 1999, Pulford et al., 2002, Vangronsveld et al., 2000) and organic compounds (Campanella et al., 2002, Carman et al., 1998, Meagher, 2000, Susarla et al., 2002). One strategy is to use fast-growing trees, particularly *Salix* and *Populus* species, to remove, stabilise or enhance the volatalisation of polluting chemicals (Greger and Landberg, 2000, Jones et al., 1999, Pulford et al., 2002, Vervaeke et al., 2002). There is a real possibility that plants can be used to reclaim contaminated land and restore sustainable and healthy soils (Dickinson, 2000, Kearney and Herbert, 1999).

Contaminated canal sediment

Environmental improvement of a derelict section of canal in Warrington was prohibitively expensive due to the cost of disposal of some 40,000 t of contaminated sediment. From an earlier survey it was known that the canal sediment consisted of a wet, black, odorous and oily mud (up to 1.5-1.7 m depth) containing a wide range of elevated contaminants including Cu, Zn, Ni, As, Pb, Cd, Cr, phenols, mineral oils and S. The proportion of bioavailable metals in this type of sediment may be as much as 40% of total, even after 60 years without disturbance. This may present a considerable hazard of dispersal to the wider environment if wet, reduced sediments are disturbed or dredged for land disposal.

The 2 km section of canal at Woolston was excavated in 1821 to improve navigation along the river Mersey by cutting out a large, looping section and hence straightening its course. A weir just after the upstream end of the canal marks the limit of the tidal reaches of the river and hence the downstream end of the canal is under tidal influence. Water flow into and out of the canal was controlled by lock gates and an aqueduct took overflow water across the river to feed into another nearby canal. Historical industrial development along the Woolston New Cut Canal appears to have been limited to a chemical works (active for at least 40 years, maybe as much as 70 or 80 years), a gunpowder mill, a tannery (only operational for perhaps a decade), an abattoir, a brick works and a piping and tubing works. These industries will have discharged into the canal and left their mark in the composition of the sediment. The original sailboats and barges which used the canal gave way to oil power and this is undoubtedly another source of pollution via leaks and spillages. Adjoining sections of the river Mersey have supported various industrial and engineering works including many metal works, more tanneries and a gas works.

Rail and road transport came to outdo canal transportation and the canal was allowed to fall into disrepair. It has not been used since the late 1940's. Perhaps associated with the demolition of the aqueduct in 1978, the canal appears to have ceased to flow. Water levels gradually dropped and the standing water and wet sediment has now been colonized by vegetation, dominantly *Typha latifolia* (Reedmace), with *Salix atrocinerea* (Sallow) at the edges.

Modern developments along the canal include housing estates, an industrial estate and a green waste recycling facility.

Aims of the research project

The project described in this paper is a case study of the feasibility of using phytoremediation *in situ* in the contaminated sediment as a low-cost alternative to cart and dump. By modeling this ecosystem, the objectives are to investigate whether metals can be rendered immobile and non-hazardous in soils and biomass, whilst plant roots and developing biota optimise conditions for the natural attenuation of organics as well as transforming the sediment into a soil.

METHODS

Site preparation

Established vegetation was cleared from a 150 m section of the New Cut Canal south bank and from shallow sediment on that side. The south bank is the shallow side of the canal, the deep side being the north side with the towpath running along the bank.

A raised platform (3.5 m wide) above existing water level was then created through dredging and transfer of sediment from the towpath side to the shallow side of the canal (Figure 1). The platform was divided into 6 experimental blocks. Twelve short-rotation coppice species, hybrids and clones of willows, poplars and alders (Table 1), were then planted on the raised platform in double rows (0.5m x 0.5 m) of 6 plants of each clone, with 1 m between rows. *Salix* and *Populus* were planted as pegs, and *Alnus* was planted as 50-70 cm rooted stock (which was pruned back after establishment). Control plots were also included in the design. Species were randomly allocated within each block.

Three sediment samples (0-15 cm) were taken with an auger between each row, and then bulked for each of the 6 blocks. The samples were thoroughly mixed and then evenly divided into four. One set was sent to each of three national UKAS accredited laboratories for analysis and the forth set was kept in house.

Five boreholes were established to the side of the canal to monitor groundwater in case of contamination. Due to the canal having a clay liner, any contaminants which may leach from the sediment bank should remain within the bounds of the canal and not cause contamination. Establishment of growth of the trees was monitored during the first year, and invasive plants were controlled by hand weeding.

Table 1. Tree species and clones planted on the raised platform of sediment within the canal.

Salix viminalis 'Jorunn' *
Salix viminalis x *schwerinii* 'Tora' *
Salix caprea x *cinerea* x *viminalis* 'Calodendron' *
Salix viminalis x *burjatica* 'Ashton Stott' *
Salix viminalis x *caprea* 'Sericans'
Salix fragilis *
Salix atrocinerea #
Populus deltoides x *nigra* 'Ghoy'
Populus trichocarpa 'Trichobel' *
Alnus glutinosa
Allnus incana
Alnus cordata

* Recommended for SRC use in FC Information Note 17. # From cuttings of trees that had naturally colonized the canal.

CURRENT PROGRESS

Contamination levels

Results of sediment analyses showed considerable variation between the three laboratories (Table 2). Part of the explanation for these differences concern different analytical methodologies – the details of which were not automatically provided in full detail by any of the laboratories. Whilst the three laboratories were UKAS accredited, standard methods for sample preparation, extraction and analysis vary (Dickinson et al., 2000). Although there is a general consensus in the determinands that exceed existing thresholds, significant differences existed between the laboratories for most determinands. The only exception was for sulphide where data were very variable between samples which probably masked differences between laboratories.

Table 2. Mean values ($\mu g\ g^{-1}$) for sediment contaminants, as provided by separate UKAS accredited laboratories Values are means of 7 samples. Shaded values are those exceeding standard thresholds of contamination.

Determinand	Laboratory		
	A	B	C
Sulphate (% SO4/SO3)	0.53	6022 (total)	0.97
Sulphide	1078	354	84.0
Arsenic	349	682*	416
Boron	69.0*	3.5	1.6
Cadmium	12.7	13.6	18.1*
Chromium	790*	1471*	977*
Copper	567*	1076*	736*
Lead	1221*	2150*	1443*
Nickel	67	78	783.1
Mercury	3.8	7.5*	4.8
Zinc	3631	5835*	4286
Cyanide	103*	10.6	23.2
Total PAH	216*	141	121
TPH	7636*	5671*	2207* (C6-C40)

* Significantly different to results of other laboratories.

Figure 1. Planting platform of dredged sediment being
prepared on the shallow side of the canal

Tree establishment

Tree establishment was very successful, with only 25% mortality in the first year. The least successful species was *S. atrocinerea* (mortality 67%). Although this species had naturally colonized the canal, it appeared to be a difficult species to root from cuttings. Survival of all other species was good.

Further research

During the course of the project we will be studying various aspects of the functioning of the ecosystem. At present a pot experiment is underway to assess the effects of various amendments on the rate of remediation. Soil fauna will be studied and its composition monitored and related to soil health.

Laboratory experiments will determine the changes in the sediment and its contaminants during drying and oxidation. The bioavailability of the metals in the sediment will also be monitored over the course of the project. We will look at the transfer of foliar metals to herbivorous animals in a laboratory environment as a model for food chain transfer.

DISCUSSION

The sediment of the canal, whilst waterlogged, provides an anoxic, reduced environment. Metals will be in reduced forms and breakdown of organic contaminants will be minimal. After dredging, dewatering and exposure to air, the sediment will start to oxidise. Oxidation will induce a host of chemical and biological changes in the sediment and the contaminants contained within it. Sediments deposited on land following dredging have been the subject of previous studies in Belgium (Tack. *et al.*, 1996, Tack. *et al.*, 1998, 1999, Tack and Verloo, 1999) and the UK (Stephens et al., 2001). Most metals show a redistribution from residual to mobile phases during drying and oxidation that is also associated with decreasing sulphide/sulphate ratio. Metal bioavailability may be particularly high in the early stages, but long-term prediction of metal migration is uncertain.

In the present project, the sediment was retained within the canal and it is likely that migration of metals to the wider environment will be controlled to a large extent by the clay liner of the canal. However, disturbance of the bottom sediment and leaching from the dredged sediment may affect contaminant concentrations in the water column of the canal. Hence, before this can be seriously considered as a treatment technology, it is important to demonstrate that contaminants are not quickly dispersed to the wider environment. Another potential source of dispersion is through food chains (Vandecasteele et al., 2002, 2003) and this will be assessed over the next two years.

I. CONCLUSION

Manipulating the processes of contaminant dispersion or immobilisation offers a real possibility of treating contaminated sediment without removal, whilst contributing to a healthy, sustainable, non-hazardous landscape of high ecological and amenity value. If successful, this will provide a realistic, generic and transferable methodology with wide application for cost-effective *in situ* reclamation of contaminated sediments.

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