Tree planting for phytoremediation:

The fate of soil contaminants on brownfield sites.

Christopher James French

BSc. (Hons) Environmental Biology. The University of Liverpool

Thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Liverpool John Moores University

This research was carried out in conjunction with The Mersey Forest and The University of Liverpool

November 2004

Biological and Earth Sciences,

Liverpool John Moores University

Liverpool L3 3AF

Dedication

To my Mum, Penny French (1948 – 2005) and my fiancée, Caroline

Both of whom supported me throughout,

Abstract

This study evaluates fast-growing woody species for phytoremediation, biomass production and community forestry on heavy metal contaminated brownfield sites in North West England. Field trials using *Salix* (5 taxa) *Populus* (2 hybrids), *Alnus*, *Betula* and *Larix* were established at six sites. Metal fluxes in plants and soils, productivity and mortality were measured over 3 years.

The heterogeneity of contamination on brownfield land proved problematic when defining baseline data, despite comprehensive sampling and mapping. UK guidance inadequately defines baseline soil contamination and sampling was carried out to identify 1% hotspots. Pseudo-total, EDTA and $CaCl_2$ extractants were used to provide a guide to soil metal bioavailability. EDTA proved most effective overall, $CaCl_2$ provided low recoveries and poor analytical detection in many cases.

Careful ground preparation, planting and stand management provided good rates of establishment of short-rotation coppice at the brownfield sites. Overall mortality rates were 10% in the first year, rising to about 20% after 3 growing seasons. Willows survived better than poplar and productivity was higher. Biomass production varied with site and taxa, but yields from *Salix* were up to 13 odt ha^{-1} yr^{-1}, making commercial use a serious consideration on 50% of sites. *Alnus incana* was also considered to be potentially valuable due to high yields.

Phytoextraction of two of the more mobile metals may be feasible. Cd and Zn were accumulated consistently in stems and foliage of *Salix* and *Populus*; the latter generally exhibited lower uptake. Uptake by the other species was generally low, with some exceptions. Concentrations of Cd and Zn in soils, stem and foliar tissue were all correlated, which may allow less destructive sampling to estimate uptake of these elements into harvestable woody tissues. EDTA-extractable soil metal concentrations did not significantly change over 3 years, despite substantial tree uptake. There was one exception, a significant decrease of soil concentrations of a range of metals was detected beneath *Salix* X *calodendron* (CAL; a likely hybrid of *S. viminalis*, *S. caprea* and *S. cinerea*). Together with *S. burjatica* Germany (GER), these taxa exhibited the ability to concentrate Cd and Zn. Stem uptake of Cd was 7-9 times (and foliage uptake was 9-13 times) higher than soil EDTA concentrations, even where soil concentrations of this metal were very low. These taxa had lower biomass yields and low mortality. Other taxa with increased yields and lower concentration factors were able to accumulate similar quantities of Zn and Cd on sites when using a mass balance model.

Metal offtake from the plots by harvest was calculated, showing significant flux of metals to wood and foliage. Leaf fall accounted for loss of over 50% of net Zn uptake, and was highest in *Populus*, which has more foliage. Harvesting before leaf fall is critical if maximum metal extraction is required. Data were also extrapolated to over a 20-year crop lifecycle. Theoretically, SRC harvest could remove 5.5kg of Cd and 150kg Zn in 20 years, representing a reduction in soil concentrations of 100-150 ppm Zn and 6 ppm Cd. It is argued this may be particularly significant for Cd on brownfield land.

Acknowledgements

In a project of this scale there are numerous people to whom I must extend thanks. Many people were involved in planting, maintenance, site works and advice. However, thanks to the following for their roles in this production.

Paul Nolan and the extended team at The Mersey Forest for stewarding the project.

The original cast of the JMU 6th floor, and latterly 1st floor labs; for coffee breaks, Christmas mulled wine and professional discussion including numerous trips to Dr. Duncans 'library'.

Tony Hutchings, Andy Moffat and other staff at Forest Research for support and sowing the initial seed to do a Ph.D.

Technical and academic staff at JMU, specifically Lou, Bev and Don for supplying everything from trowels to tea. Prof. Nick Lepp for his advice and support. Prof. Rob Marrs (UoL) for statistical advice.

Gary Noble, John Roles, Eugene Pierce and Steve Holmes exhibited extreme fortitude and hard work as sandwich students working on this project. Sincere thanks for their many hours of hard work in the field and the lab (and bar).

Caroline Richardson and Kate Jacobs, two outstanding housemates and friends to whom I owe a great deal.

Nick Dickinson and Phil Putwain for supervision, patience and friendship despite their wayward, globe trotting student.

The assembled masses of the family French and family Stokes.

Finally and most importantly, my fiancée Caroline, for being the best distraction from work and supporting me in every way throughout the whole production.

CJF. November 2004

Glossary / Experimental Definitions

This section should be used for reference in conjunction with textual descriptions. Site and species definitions and abbreviations are described fully in the text and summarised here.

Study Specific Definitions

Heavy Metal – Chemical notation defines them as 'elements with an atomic density greater than 6 g/cm^3. The term however, has become synonymous with elements that are potentially toxic in the environment.

Taxa – Any named taxonomic group of any rank in the hierarchical classification of plants. In this study, taxa specifically refer to morphologically different varieties of *Salix*.

Stem – refers to above ground woody parts of the tree, including bark

Foliar / foliage – leaf and petiole material

Wood / Bark – constituent parts of stem

General Terms

AAS – Atomic Absorption Spectrophotometery

CLEA – Contaminated Land Exposure Assessment

CRM – Certified Reference Material

DEFRA – Department of the Environment, Food and Rural Affairs

DGT – Diffuse Gradients in Thin Films

EA – Environment Agency

EDTA – Ethylenediaminetetraacetic Acid

ICP-OES – Inductively Coupled Plasma Optical Emission Spectrometry

ICRCL – Interdepartmental Committee on the Redevelopment of Contaminated Land

JMU – Liverpool John Moores University

ODT ha^{-1} – Oven dry tones per hectare

PCA – Principal Component Analysis

SGV – Soil Guideline Value

SRC – Short Rotation Coppice

UoL – University of Liverpool

USEPA – United States Environmental Protection Agency

Experimental Site abbreviations (refer to section 2.1 for a full description)

COR – Deeside Steelworks (Corus)

CRM – Cromdale Grove

FAZ – Fazakerley Reed Bed Scheme

KIR – Kirby Moss

MER – Merton Bank

SUG – Sugar Brook

Experimental Species abbreviations (refer to section 3.3 for a full description)

LAR - *Larix x eurolepis*

BET - *Betula pendula*

ALN - *Alnus incana*

GHY - *Poplus deltoides x nigra* 'Ghoy'

TRI - *Poplus trichocarpa* 'Trichobel'

CAL - *Salix caprea x cinerea x viminalis* 'Calodendron'

ORM - *Salix viminalis* 'Orm'

CLS - *Salix caprea x viminalis* 'Coles'

GER - *Salix burjatica* 'Germany'

TOR - *Salix viminalis x schwerinni* 'Tora'

Contents

List of Tables

List of Figures

List of Plates

List of Equations

Chapter 1: INTRODUCTION

1.1 Introduction

The concept of phytoremediation is not new. In 1885 a paper was published noting the ability of green plants to concentrate and accumulate inorganic metallic elements (Baumann 1885). In a present day context phytoremediation is generally defined as "The use of vegetation to contain, sequester, remove, or degrade organic and inorganic contaminants in soils, sediments, surface water, and groundwater" (Schnoor 1997). The increased problem of anthropogenic organic and inorganic pollutants within terrestrial and aquatic ecosystems has transformed a 19th century botanical curiosity into a remediation technology aimed at manipulating and ultimately reducing the effects of harmful contaminants in the environment.

The background to, and rationale of, the present work examines the interactions of different tree species planted for phytoremediation on Brownfield sites contaminated with a range of heavy metals types at differing concentrations. It considers many characteristics of growing trees in such environments and discusses the wider concept and benefits of applying such schemes in a community forestry or commercial production context, within current legislation and statutory controls in the UK.

Previous experiments have examined the phytoremediation potential of *Salix* (Willow) and other tree species in relation to heavy metal contamination, but have concentrated predominantly on pot, nursery or limited field experiments. Those that have taken place in the field have favored sites contaminated with heavy metals associated with sewage sludge, fertilizer residues, or their own unique soil chemical environments. This study aims to examine brownfield sites with a range of historical contamination sources created though different processes and timeframes.

1.2 Brownfield Land

Brownfield land is a widely employed term applying to land that has previously been used in a productive context and now lies dormant with no perceived beneficial use. Definitions vary widely, and other terminology such as derelict land, vacant land and underused land are employed to refine descriptions of brownfield land, mainly for the benefit of statutory definitions. An important distinction is that not all brownfield land is contaminated and, conversely, not all contaminated land is classed as brownfield. Many contaminated sites may still be part of current industrial operations, parks, greenspaces or residential areas.

In 2002 an Environment Agency report stated:

"Brownfield and derelict sites, and even some greenfield sites, may be affected by contamination, and may, or may not, meet the statutory definition of contaminated land"(Environment Agency 2002).

The point at which sites can be classed as contaminated depends on current UK statutory designation of contaminated land, however, many brownfield sites may contain contaminants at levels below the statutory definition, but still acting as a barrier for site development or re-use. One of the main reasons for the emergence of brownfield land is economic and structural change, with decline of traditional industries. In many cases such sites are usually accompanied by severe job losses, and a spiral of social and neighborhood decline (Grimski and Ferber 2001). This situation places a much higher emphasis on returning brownfield sites to a beneficial use, even as an interim solution.

1.2.1 Regional Context

In 2002 the Environment Agency estimated that North West England contained 6,930ha of derelict or vacant land, the second largest regional quantity in the United Kingdom. (Environment Agency 2002).

This figure is predominantly a result of the North West's heritage of heavy industry, such as chemical production and mining in the 19^{th} and 20^{th} Centuries that fueled the industrial revolution in the UK. Recently brownfield land has been generated by the subsequent cycle of urban sprawl and the dereliction that followed the demise of these large industries.
Over time this socio-economic shift has created significant areas of brownfield land, much of which is located in urban areas, which expanded with associated industries and now most of which have long ceased production. Many sites, by the nature of their location also acted as formal and informal areas of industrial waste disposal and have an unknown pollution history as few records were kept of substances used or dumped. Irrespective of the actual contamination issues surrounding these sites, their location and perception are sufficient that there are very few drivers to either remediate or re-develop them.

The concept of urban renaissance is therefore receiving renewed interest from national and local government. The transformation of post-industrial brownfield sites to soft end uses such as amenity land, parks or forestry is seen as critical in dealing with a range of urban problems relating to health, environment and social inclusion (Barton 2000). Although significant attention is still being given to the reuse of brownfield land for new homes or other commercial hard end uses, in areas such as the North West, there is neither the demand for homes in certain areas, nor the funds for redevelopment or remediation.

Understanding the regulatory and statutory framework for such sites, as well as the physical site constraints will help to make sure that community forestry and similar soft end use schemes succeed in the long term.

1.3 Contaminated Land

In 2000 the UK received its first statutory definition of contaminated land under new legislation inserted into part IIA of the 1990 Environmental Protection Act;

Contaminated land is defined at section 78A(2) as:

> "any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land, that -
> "(a) significant harm is being caused or there is a significant possibility of such harm being caused; or
> "(b) pollution of controlled waters is being, or is likely to be caused; ..."

Further definitions of significant harm, and the significant possibility of significant harm to receptors are also defined. Receptors are listed as humans, ecosystems (classified as SSSI's or above), livestock or crops and buildings. Any receptors not included in this list are disregarded for the purposes of statutory definition. This means a clear pollution linkage pathway must be established from the source to one of the above receptors for the land to be deemed as contaminated. Site specific assessments must be made based on end uses for the site and potential exposure pathways. Sites comprising a sensitive land use, such as schools, housing or allotments maybe defined as contaminated as they have sensitive end uses and clearly defined receptors, but the same site used for a car park may not be classed as contaminated.

This definition has several major repercussions as to how brownfield land is designated and subsequently used. Some brownfield land that contains contaminants will not be defined as contaminated as no suitable receptor will be impacted. Removing this stigma will not necessary remove the contamination and may actually hinder funding and momentum to clean up or re-use the land will be drastically reduced.

Another interpretation would be to ensure that land does not become defined as contaminated by the introduction of remediation schemes that will cause receptors to be open to contamination. In the case of tree planting for example, would trees provide a pathway for metals to enter food chains or become more mobile in the soil, thereby introducing a contaminant transport pathway and potentially defining the site as contaminated?

1.3.1 Estimates of the quantity of Contaminated Land

Estimates of contaminated land in the UK are varied due to differing interpretations and applications of definitions, so should be viewed sceptically. In 2000 the Environment Agency estimated there was 50,000 to 200,000 ha of contaminated land in England, representing approximately 100,000 sites of which between 5,000 and 20,000 may require remediation based on unacceptable risks to human health and the environment (Environment Agency 2000). In 2002 a revised methodology assessed the number and area of sites previously used by industries that had the potential to cause contamination. In the North West this revealed 20,000 sites covering nearly 35,000ha that had been subjected to potentially contaminative processes (Environment Agency 2002). This was the highest regional number of sites in the United Kingdom.

Although it is likely that only a small proportion of these sites will represent a direct danger to humans or the environment, it shows the large potential for contaminated sites based on the regions industrial heritage.

1.3.2 Regulatory Control of Contaminated Land

Assessment of contaminated land is now controlled by Contaminated Land Exposure Assessment (CLEA), a human health risk based system since that was introduced to compliment new statutory guidance (DEFRA and Environment Agency 2002a). Previous guidance used to describe if land was contaminated and therefore its suitability for a range of end uses, were concentrations specified by the Interdepartmental Committee on the Redevelopment of contaminated land (ICRCL 1987). These figures were designed to be used as guidance for hard end redevelopment and consisted of a series of trigger values and threshold limits, split into predominately zootoxic and phytotoxic elements, although definitions also were noted for some organic compounds. Figures for metals were in part derived from the use of sewage sludge in agriculture (McGrath 1994).

It is a widely held claim that these (ICRCL) figures for the UK were 'inadequate and... lacking entirely in relation to tree planting'(Dickinson *et al.* 2000), and ICRCL figures were never written into any UK statutory definitions. A variety of other national and international guidelines were historically used such as Kelly indices or the Dutch list, none of which had any statutory or risk based derivations. Consideration of the CLEA and ICRCL guidelines in relation to the remediation of contaminated brownfield sites is discussed at length in Chapter 4.

1.3.3 Defining and classifying Contaminated Land

Site investigation is a basic requirement for quantification, remediation and risk assessment of contaminants, which in turn may affect any future uses of the land, especially where public access is required or when controlled waters or ecological receptors are impacted. Intrusive site investigations involving extensive soil or groundwater sampling and subsequent analysis are required to accurately assess spatial variability of contaminants. This may reveal important data otherwise masked by the descriptive statistics of desk studies and more restricted site investigations.

Contaminant sampling on sites with poorly documented historic contamination is problematic in comparison to areas with better defined sources of pollution. If the contamination source were more readily identifiable, such as a leaking tank or surface water flow path, spatial patterns of contamination could be more readily quantified. It is easier to evaluate sites known to have been amended with sewage sludge, affected by atmospheric fallout from industrial stacks, or impacted by mining waste disposal. In these cases the footprint and direction of contamination is likely to be more clearly defined. Previous studies of contaminated sites and metal uptake by plants have focused on these types of sites where the substrate is better defined and of a less heterogeneous nature. Issues surrounding investigating and mapping contamination are discussed in Chapter 4.

1.4 Sources of Heavy Metal Pollution

Metal pollution can be released into soils in large amounts via natural weathering processes of parent rock materials (Borovik 1989). Sedimentary rocks such as clay and shale contain higher quantities of metal ions from absorption (Brooks 1987). These rocks are faster weathering and soils formed upon them may have naturally elevated levels of heavy metals. Other natural sources of heavy metal input to the biosphere are from volcano eruptions and to a much lesser extent, forest fires.

The main anthropogenic activities causing metal entry to soil and atmosphere are (Alloway 1995b):

- Metalliferous mining, refining and smelting
- Agriculture and sewage sludge disposal
- Combustion of fossil fuels
- Metallurgical and specialist industries
- Disposal of waste material.

Metal contaminants considered by these processes are Ag, As, Au, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Se and Zn. In terms of quantities released and distribution in the environment the most significant are Cd, Cu, Zn, Pb and Ni (Alloway 1995b).

Their source of entry to ecosystems is dependant on use, for example via metalliferous aerosols from chimney stacks, from mining waste spoil heaps or disposal of contaminated waste materials. In many areas more than one source may be present, and a lack of accurate records concerning previous uses or wastes can make pinpointing sources difficult.

1.5 Heavy metals in the soil

The conditions of the soil into which heavy metals have been deposited will have a substantial effect on their behaviour, fate and ultimately interaction with plant systems. Physical and chemical soil factors such as pH, organic matter concentration, nutritional status and particle size distribution will affect the behavior of metals present in the soil (Ernst 1996). The nature and chemical form of metal contaminants, the presence and concentration of other elements and abiotic factors such as weathering of minerals and groundwater hydrology will also affect contaminant behaviour. Further biotic influences such as the interaction of plant roots, soil bacteria and fungi and soil fauna such as earthworms or other macro invertebrates can affect soil contaminant behaviour. It is the vast number of physical, chemical and biological interactions that make modelling and predicting the availability, fate and transport of these metals within the soil-plant system complex.

Soils may contain naturally derived heavy metals, or elevated concentrations due to anthropogenic inputs. Background levels of metals in soils in an undisturbed state and soils from urban environments have been well documented (Angelone and Bini 1992; McGrath and Loveland 1992; Dickinson *et al.* 2000; Kabata-Pendias 2001).

The contaminants of concern to be investigated in this study are described below:

1.5.1 Arsenic (As)

Arsenic is primarily described as a metalloid, although its fate and behavior in soil is more akin to a non metal covalent compound, forming complex ionic species. Arsenic may occur naturally in many soils as a result of mineralisation and weathering processes (Mitchell and Barr 1995). Anthropogenic inputs of As may derive principally as a by product from the processing and extraction of ores such as copper, lead, tin and silver (Kavanagh 1997), atmospheric deposition from smelting, waste materials from the glass, tanning, electrical and ceramics industry and use of agricultural pesticides or timber treatment chemicals (O'Neill 1995).

Arsenic may exist in the soil in either the +5 oxidation state, Arsenate As(V), typically found in normal aerated soil, or the 3+ valence state, Arsenite As(III) which is more stable and found under waterlogged or anoxic conditions. Typically 90% of dissolved As found in aerobic soils is found as As(V), although this can reduce to as little as 15% in waterlogged conditions in favour of the As(III) form (Wasay *et al.* 2000). Organic arsenic can also be present in soil, but inorganic forms are the most toxic (Pongratz 1998). Arsenic speciation will be influenced by pH and microbial activity, as well as the presence of clays, Fe and Al oxides and soil organic matter to which both As forms adhere. Arsenic is carcinogenic and genotoxic to humans via oral and inhalation routes (DEFRA and Environment Agency 2002c).

1.5.2 Cadmium (Cd)

With the exception of a small number of carboniferous black shales, natural occurrence of Cd in the soil by mineral weathering processes is extremely low, typically less than 1mg kg^{-1} (Alloway 1995a). Anthropogenic sources consist of mining (as a by product from Zn smelting) and waste disposal activities, either of products containing cadmium such as batteries, plastics and galvanized metals or aerial deposition from smelting, burning fossil fuels and tyre ware (DEFRA and Environment Agency 2002f). On agricultural, or former agricultural land, Cd contamination in soil occurs as a result of phosphate fertilizer use, where Cd is present in rock phosphates. This may be exacerbated by the use of sewage sludges on soils, which frequently contain high levels of Cd. Cadmium is most commonly found in the soil as free Cd^{2+} ions and is therefore one of the most mobile heavy metals, although it may be adsorbed onto clay or mineral surfaces and adsorption increases with soil pH. Cd is primarily zootoxic and represents a risk to human health via oral and inhalation pathways, the latter representing and carcinogenic and genotoxic risk (DEFRA and Environment Agency 2002d).

1.5.3 Copper (Cu)

Elevated Cu concentrations may be found in the soil surface as a result of smelter deposition or mining wastes. On agricultural land Cu inputs may originate from fertilizers, pesticide use, the use of sewage sludge, or litters and manures from animals fed with Cu compounds. Cu strongly associates with organic matter and is the primary source of its retention within the soil, but may also complex with Fe and Mn oxides and silicate clays. As an essential plant micronutrient Cu is utilized as an activator and also in some enzyme systems, although absorption into plants is among the lowest of plant essential elements. Increased quantities of Zn^{2+} ions are antagonistic to Cu uptake as the mechanisms are thought to be similar. Cu in excessive concentrations is known to be phytotoxic. The main factor controlling Cu retention and mobility in the soil is the quantity of organic matter, Cu forms stable ligands with COO- groups in the solid and liquid phases of organic matter. This has been identified as the cause of Cu deficiencies, but also in the decrease of Cu toxicity in high organic matter soils (Baker and Senft 1995).

1.5.4 Nickel (Ni)

Nickel is present in many ores and ultramafic igneous rocks such as sulphates and silicates. Mean concentrations of Ni in soil were reported to be 20mg kg^{-1} (McGrath and Loveland 1992). Nickel is commercially mined from sulphide and oxide ores and is commonplace in many metal alloys, platings, batteries and chemical catalysts. Atmospheric Ni deposition is via ore smelting or burning of fossil fuels including oil and diesel. Direct application of Ni to soils is primarily in the form of disposal of fly ash from coal fired power stations, followed by application of sewage sludges (DEFRA and Environment Agency 2002g). Ni is a transition element and is commonly found in soils as a divalent ion, Ni^{2+}. Ni(II) is stable under a range of pH and redox soil situations and increased alkalinity causes Ni to form hydroxy complexes (McGrath 1995). pH is the most important factor for Ni distribution between soil and solution phases and for plant uptake (Kabata-Pendias 2001), although binding of Ni to organic matter is also significant. Ni is recognized to be phytotoxic at concentrations from 10-100 mg kg^{-1} depending on plant species.

1.5.5 Lead (Pb)

Lead is a component of igneous rocks and forms sulphide ores. Soil is know to act as a sink for anthropogenic lead of which the primary sources are mining, smelting, sewage sludge and aerial deposition from vehicle exhausts (Davies 1995). Lead is used in batteries (60% of usage), metal products and historically in paints. In the late 1980's it was estimated that anthropogenic emissions of Pb were more than 20 times greater than natural emissions, far greater than any other trace metal (Nriagu 1989). Consequently atmospheric deposition is the major input into the biogeochemical cycle, especially in urban areas, although this was substantially reduced with the phasing out of lead in petroleum products (Watmough and Hutchinson 2004). Lead in soil occurs in Pb(II) and Pb (IV) oxidation states both of which are stable, and typically accumulates in the top of soil profiles (Davies 1995). Much is known about human health effects of increased exposure to lead which can result in reduced cognitive development in children and reduced sperm counts (DEFRA and Environment Agency 2002b). In soils with a pH range of 5.5 – 7.5 lead solubility is controlled by phosphate and carbonate precipitates thus very little is available to plants (Blaylock *et al.* 1997).

1.5.6 Zinc (Zn)

Burning fossil fuels, metal smelting and sewage sludge are all anthropogenic sources of Zn. Zinc is extremely mobile and bioavailable in soil, existing predominantly as a divalent cation below pH 7.7 (Kiekens 1995) and is adsorbed mainly onto organic matter and clay minerals. Zinc commonly interacts with Phosphorus in the soil system, and high P may result in a decrease in Zn uptake and availability. Due to its high bioavailability plant tissue concentrations generally rise with zinc soil concentrations.

1.6 Uptake of metals by plants

Plants require mineral nutrition for optimum growth and metabolic activity. These can be grouped in essential macronutrients, essential micronutrients and those having only beneficial or restricted use. All plants require essential macronutrients; N, P, K, S, Ca and Mg in large quantities, typically 1000 mg kg^{-1} or more dry weight. Essential micronutrients are limited to just 10 trace metals; Fe, Cu, Mn, Zn, Co, Mo, Ni, V, Na and Rb (Kieffer 1991) at concentrations equal to or less than 100 mg kg^{-1} dry weight (Mehra and Farago 1994). Al, Sn, Cr and Sr are recognised as being beneficial to plants. Elements such as As, Ag, Cd and Pb may be required at extremely low concentrations but generally serve no recognised biological function and can be toxic at higher concentrations.

The mechanisms used to translocate and utilise nutrients are the products of several millennia of evolution. Such mechanisms are selective, acquiring some metals in preference to others.

In order to solubilise and extract metal ions from the soil environment plant roots can modify the soil environment in several ways by exuding protons to change soil pH, or exuding other compounds such as amino acids and hydroxycarboxylic acids to complex trace metals (Cataldo *et al.* 1987). Soil biochemical activity in the rhizosphere, stimulated especially by carbon based plant root exudates, can mobilise metals though acidification and organic complexation (Pahlsson 1989). Conditions within the rhizosphere can therefore differ dramatically from the bulk soil component due to these rapidly changing and dynamic processes.

Absorption of metals directly into the leaf can occur as a result of deliberate deposition (such as the use of fertilisers) or accidental deposition (such as anthropogenic aerosol sources). Species type, leaf age, cuticle thickness and position and nutritional status will all influence the rate of foliar absorption (Alloway 1995b). In many cases the waxy cuticle will prevent translocation into the leaf, especially in the case of Pb, and metals become bound into the leaf surface. Metals in solution may penetrate the leaf directly though the stomata where they can be transported to other parts of the tree via the phloem. Aerial deposition may also increase soil metal levels as they are washed down the trunk and accumulate in the soil around the base of the tree (Watmough and Dickinson 1995).

1.6.1 Fate and distribution of metals in trees

The fate of metals as they pass from the soil to plant system varies widely across species and metal type. Sycamore trees grown on contaminated soil showed the deposition of lead not retained within the roots was accumulating to the stem, whereas zinc tended to be found within the leaves (Turner and Dickinson 1993). Other studies have suggested that metals accumulate more rapidly in actively growing parts of the tree such as shoots and leaves. Riddell-Black (1994), grew four willow species on soil treated with repeated applications of sewage sludge and demonstrated higher concentrations of all metals in leaves then of those in the stem. Rosselli *et al.* (2003) grew *Salix* and *Betula* species on contaminated material to find significantly higher concentrations of Zn and Cd in leaves and shoot tissues than in the stems, similar findings were repeated with a related experiment using *Salix* in pot experiments by the same authors. Similar compartmentalisation patterns were also noted by Punshon and Dickinson (1997), where Cu and Pb accumulated predominantly within the stems, and Zn and Cd in the leaves, although considerable concentrations of Cd and Zn were also present within the stems.

Ultrastructual studies conducted on plant material using transmission electron microscopy (Jarvis and Leung 2002), observed the distribution of both chelated and unchelated lead in *Pinus* species. Unchelated lead was found exclusively in cell walls of the roots, whereas lead chelated with EDTA was found in intracellular spaces and sites adjacent to cell walls in the needles, with no apparent effect on the morphology of intracellular components.

1.7 Inducing changes via plants in the soil metal bioavailability

Section 1.6 discusses the ability of some plants to manipulate soil metal availability through exudation of hydrogen ions or other compounds. This manipulation may increase metals available for plant uptake, or reduce metal mobility and thus migration either off site or into plant tissue depending on the remediation strategy desired, eg: phytoextraction or phytostabilisation.

Nissen *et al.* (2000) used synthetic zeolites to amend a sewage sludge based compost and found that labile pools of zinc were significantly reduced, so was soil to plant transfer and proved as effective as using larger quantities of lime as an amendment. Chen *et al.* (2000) conducted similar experiments on rural Cd and Pb contaminated soils. The use of Calcium Carbonate, Manganese Oxide and Zeolite, reduced Cd and Pb availability in soils and significantly reduced uptake into wheat shoots compared to controls.

A different strategy can be adopted in order to increase metal availability for remediation methods such as phytoextraction, especially for metals such as Pb and Cd, where uptake rates may be low. This is especially true of lead, a ubiquitous contaminant in environments, with a low bioavailability in the soil and a poor ability to transport lead from roots to shoots. Blaylock *et al.* (1997) used EDTA to enhance the availability of Pb to Indian Mustard (*Brassica juncea*), resulting in concentrations of 1.5% Pb in the shoots. Similar results were obtained by Huang *et al.* (1997) using almost identical methodologies.

In laboratory tests, Lim *et al.* (2004), augmented soil EDTA addition by applying electric currents to soil surrounding plants to further increase extraction using *Brassica juncea* by a factor of 2-4 compared to using EDTA alone, although such systems may have limited use in field applications. Lai and Chen (2004) concluded the plant *Dianthus chinensis* increased Pb uptake from a soil amended with EDTA, although soils in this trial were artificially contaminated and represent a poor field comparison.

Robinson *et al.* (2000) used EDTA, DTPA and NTA in a pot experiment to increase Cd availability to *Populus*, although the use of EDTA and NTA led to a reduction in growth and abscission of leaves.

Consideration of soil conditions and potential leaching of mobilised metals to a wider environment must be made when applying chelating substances, and similar drawbacks to this technique do exist. Increased rainfall may lead to faster leaching into the environment and EDTA addition may cause leaching of essential macronutrients such as Fe from the soil. The resulting poor plant growth and therefore uptake will further risk contaminated leachate migrating to groundwater (Wu *et al.* 2004). Implementation at a field scale must minimise leaching and the application of more than 1 g/kg EDTA may become inefficient as lead concentration in crops was not enhanced and leaching rate increased (Schmidt 2003). A recent, comprehensive review of assisted extraction technologies is presented by Schmidt (2003).

1.7.1 Alternative mechanisms of altering soil metal bioavailability

Mycorrhizae

There is a general assumption that many plant species will develop mycorrhizae under natural field conditions. These associations have evolved to increase the supply of nutrients to the plant, but may also allow the increased absorption of heavy metal ions into the roots by increasing their availability within the soil through phytochelators (Khan *et al.* 2000). Mycorhizal associations can also contribute to phytoremediation by inducing increased bacterial activity in the rhizosphere to stimulate degradation of organic products (Meharg and Cairney 2000), or to increase metal tolerance of trees and protect them from toxicity (Wilkinson and Dickinson 1994; Van Tichelen *et al.* 2001; Martino *et al.* 2003).

Soil Fauna

Soil organisms such as earthworms and soil bacteria can also influence soil metal concentrations and availability. Ireland (1975), found that *Dendrobaena rubida* altered the solubility of Pb, Zn and Ca in reclaimed Welsh mining spoils, with a 50% increase in availability of water-soluble Pb in faeces. Devliegher and Verstraete (1996, 1997) found that copper, chromium and cobalt availability were raised by between 6-30% in the soil by the earthworm *Lumbricus terrestris*. Ma *et al.* (2003) discovered the earthworm *Pheretima guillelmi* was able to increase metal and nutrient availability in soils containing Pb and Zn mine tailings, consequently increasing metal uptake into plants and improving conditions for phytoextraction. Other studies have demonstrated that earthworms can survive at high concentrations of metal contaminated soils (Langdon *et al.* 1999; Langdon *et al.* 2001). Soil invertebrates such as earthworms improve physical soil structure and fertility by increasing organic matter and reducing compaction, problems frequently encountered on brownfield sites.

1.7.2 Effects of Metals on trees

Once inside plant cells heavy metals can inhibit the growth and reproduction of plants in a number of ways. In general heavy metals can seriously disrupt many physiological processes due to their ability to bind strongly to enzymes and induce deficiencies by substituting for essential metals on metalloproteins (Van Assche and Clijsters 1990). The most pronounced damage may be in root systems where metals are first encountered and take the form of elongation or biomass reduction.

1.8 Phytoremediation

Traditional decontamination of heavy metal polluted soils frequently involves immobilization, chemical treatment or removal of large quantities of soil material. These techniques are costly, impractical for certain sites and in most cases unsustainable with contaminated material excavated and removed to landfill sites. Such practices will become considerably more expensive and prohibitive after the introduction of EU landfill directive legislation to the UK in July 2004 (IEMA 2004). In addition 'dig and dump' methods for contaminated soil treatment cause considerable degradation of the soil ecosystem, as soil biological activity is removed as new inert soil or fill material is imported. Other biological remediation methods such as the use of bacterial degradation in bioremediation and natural attenuation, are more suited to organic contaminants and have very limited uses for the remediation of heavy metal contaminated soil.

Phytoremediation can be defined as "The use of vegetation to contain, sequester, remove, or degrade organic and inorganic contaminants in soils, sediments, surface water, and groundwater". Such techniques offer a considerably lower cost, in situ and unobtrusive remediation option. It is possible to subdivide phytoremediation process based on their modes of action, media in which they can be used and contaminants effected. This is shown in Table 1.1.

Table 1.1. Phytoremediation processes (adapted from (Schnoor 1997))

Application	Media	Contaminants
Phytotransformation (including phytovoltalisation)	Soil, Groundwater, Landfill leachate, Wastewater (land application)	• Herbicides • Aromatic (BTEX) • Chlorinated • Nutrients (NO_3, NH_4) • Ammunition wastes
Rhizosphere Bioremediation (phytostimulation)	Soil, Sediments Wastewater (land application)	• Organic Contaminants (e.g. pesticides, aromatics, PAH's)
Phytostabilisation	Soil and Sediments	• Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) • Hydrophobic organics (PAH, PCB's, PCP's DDT)
Phytoextraction (Phytoaccumulation)	Soil, Brownfields, Sediments	• Metals (Pb, Cd, Zn, Ni, Cu)
Rhizofiltration	Groundwater, Wastewater in lagoons and created wetlands	• Metals (Pb, Zn, Ni, Cu) • Radionuclides • Hydrophobic organics

Phytotransformation (inc. Phytovolatilisation)

The uptake of organic and nutrient contaminants from soil and groundwater and transformation by plants. The most common form is phytovolatilisation where chemicals are released to atmosphere through plant transpiration.

Rhizosphere Bioremediation (Phytostimulation)

Bacteria and mycorrhizal fungi that are able to metabolise contaminants are increased within root zones of certain plant species. Plant root exudates, enzymes and a build up of organic carbon increase microbial populations and thus degradation rates. Oxygen is available at the rhizosphere ensuring aerobic transformations.

Phytostabilisation

Contaminated soils and sediments are held in place by vegetation, immobilising contaminants within the soil. Vegetation prevents removal of contaminants in topsoil via windblow and may also be used to control leaching of contaminants to groundwater via plant transpiration streams.

Phytoextraction

The use of metal accumulating plants to translocate and concentrate metals from contaminated soils or waters into above ground plant biomass such as shoots, bark or leaves. Metals must be bioavailable for the process to be effective.

Rhizofiltration

Plant roots are used to concentrate and precipitate metal contaminants from surface or groundwater.

Summary

Unlike organic contaminants, heavy metals cannot be degraded or destroyed. The processes of phytoextraction (phytoaccumulation), phytostabilisation and potentially phytotransformation (phytovolatilisation) are therefore the most relevant to the remediation of heavy metal contaminated land (USEPA 1998).

1.8.1 Hyperaccumulation

Considerable interest has been shown in plants that are able to accumulate metals at very high concentrations from metal contaminated soils. Defined as hyperaccumulators they are able to retain metal concentrations an order of magnitude, or 0.1% in dried leaves, greater than normal species growing on similar sites in their natural habitats (Reeves 1992). This criterion was considered appropriate for Cu and Pb, although for Zn a criterion for 1% in dried material was suggested due to increased background concentrations (Reeves and Brooks 1983).

These traits have, as at the year 2000, been identified in 417 taxa spanning 80 families, as summarised in Table 1.2

Table 1.2. Number of metal hyperaccumulating plants from Baker et al. *(2000)*

Metal	Criterion (% in leaf dry matter)	No of Taxa	No of families
Cadmium	>0.01	1	1
Cobalt	>0.1	28	11
Copper	>0.1	37	15
Lead	>0.1	14	6
Manganese	>1.0	9	5
Nickel	>0.1	317	37
Zinc	>1.0	11	5

Distribution of Ni hyperaccumulators correlate strongly with areas with soils derived from ultramafic rocks, typically containing 0.1 – 1% Ni. A common zinc hyperaccumulator, *Thlaspi caerulescens,* can actively seek zinc containing materials on heterogeneously contaminated land (Haines 2002) and accumulates metals without the aid of root exudates to enhance mobilization of metals in the soil (Zhao *et al.* 2001).

Absent from Table 1.2 is Arsenic. Recent discoveries of a potential Arsenic hyperaccumulator were noted in 2001 when the fern *Pteris vittata* L. was observed to accumulate As into plant biomass (Ma *et al.* 2001). Many avenues of additional research ensued, for example to examine metal speciation within the plant (Zhang *et al.* 2002), rhizosphere interactions (Fitz *et al.* 2003) and spatial distribution (Bondada *et al.* 2002). All these studies concluded that the fern held excellent hyperaccumulation prospects, with recent work stating that using it to clean up a US contaminated 'superfund site' to safe levels (40 $mg.kg^{-1}$ As) could take just 8 years (Salido *et al.* 2003). *Pityrogramma calomelanos* shares similar characteristics with *Pteris vittata,* and can also accumulate large concentrations of As into the above ground fronds recorded at 8350 mg kg^{-1} As dry mass (Francesconi *et al.* 2002).

Hyperaccumulators can remove metals from low and high soil concentrations and in many cases above ground plant biomass concentrations exceed those in the roots. Several theories for this trait have been postulated, such as protection of perennial root systems, a mechanism of excreting metal from plant systems through leaf fall or acting as a defense mechanism to fungal and insect attack (Pollard and Baker 1997).

1.8.2 Use of hyperaccumulators in phytoremediation

The scope for using hyperaccumulators to effect biological clean up has been recognised for 20 years (Chaney 1983), and more recently within the scope of phytomining (Anderson *et al.* 1999). However, hyperaccumulator plants usually grow to a relatively small biomass, naturally limiting any potential metal uptake. Considerable variation can exist in natural populations and growth may be increased with nutrient addition. The geographical distribution of many plants is restricted and population sizes are small, for example nearly all Cu hyperaccumulators are confined to the Democratic republic of Congo and northwest Zambia (Baker *et al.* 2000).

It may be possible to combine hyperaccumulation with increased biomass, for example in the tall herb *Berkheya coddii*, but the geographical range in this case is still limited to South Africa (Robinson *et al.* 1997), and in many cases it is still necessary to use soil amendments and chelators to improve uptake rate and quantities (Robinson *et al.* 1999). Further studies have investigated the extraction of metal from the wood of these plants for re-use (le Clercq *et al.* 2001).

Considerable research into hyperaccumulators is proceeding on a genetic and bioengineering foundation (Dushenkov *et al.* 2002), with the notion of introducing a hyperaccumulator genetic trait into a high biomass producer such as *Miscanthus* (Arthur *et al.* 2000).

1.8.3 Use of Transgenic plants in Phytoremediation

Developments in genetic modification (GM) of plants may offer an attractive route to improve phytoremediation potential. Ideal plant properties for phytoextraction are high biomass and metal uptake with a rapid growth rate and tolerance to heavy metals. Phytoremediation processes may also be enhanced by either inducing over production of plant produced chelates to increase metal availability in the soil, or increased production of metal transporter proteins (Pilon-Smits and Pilon 2002). Other potential genetic mechanisms for exploitation include introduction of bacterial degradation pathways, a technique that has been successful in improving Hg volatilisation and tolerance (Pilon-Smits and Pilon 2000; Ruiz *et al.* 2003), and is being investigated with a view to insertion into *Salix* and *Populus* species (Rugh 2000).

Research in this area is widespread, with genetically enhanced phytoremediation of Selenium (Berken *et al.* 2002), Lead (Gisbert *et al.* 2003), Arsenic (Dhankher *et al.* 2002), Zinc and Cadmium (Klein *et al.* 2002) all being actively researched. This complex subject is beyond the scope of this study, but excellent reviews on the genetic basis of hyperaccumulation in plants (Pollard *et al.* 2002), genetic metal tolerance in plants (Macnair 1993) and the use of transgenic plants in phytoremediation (Pilon-Smits and Pilon 2002), are available. Although it may be possible over time, to successfully introduce a GM plant able to remediate metal contaminated soil effectively, there are many obstacles to overcome in terms of risk assessment and public uncertainty surrounding GM based phytoremediation (Wolfe and Bjornstad 2002) given the wider global debate on the use of GMO's.

1.9 Willows

1.9.1 Background and uses of Salix

Salix have long been recognised as a pioneer species for a wide range of ecosystems (Sommerville 1992). The ability of taxa such as *Salix caprea* and *Salix cinerea* to exploit unusually harsh environments, such as nutrient poor mine spoil sites (Eltrop *et al.* 1991); (Kahle 1993) has led to the use of *Salix* in ecological restoration situations such as community forestry, bank and slope stabilisation or production of natural screens. *Salix* possesses other attributes that make them desirable in reclamation and specifically bioremediation situations, such as rapid growth and the ability to produce a large amount of biomass with a range of end uses. The predisposition for considerable morphological and ecological variation within the genus is valuable, and use of *Salix* as a remediation tool is a natural progression.

Coppicing of *Salix* to utilise the wood product is a practice dating to the earliest civilisations. It has been grown commercially for 20 years in Scandinavia as a biofuel (Rosenqvist *et al.* 2000) using specially bred biomass clones. Recent changes in UK agricultural practice, in tandem with a review on sustainable energy policy has made short rotation willow coppice a potential viable crop for renewable energy generation via biomass and co-combustion power generation facilities. This topic is discussed in detail in Chapters 6 and 7.

1.9.2 Phytoremediation possibilities of *Salix*

The use of *Salix*, and to a lesser extent poplar, for the remediation of heavy metal contaminated land has been developed for the past 15 years. The majority of research currently underway can be broadly categorised into five differing scenarios based on the substrate(s) requiring remediation, specifically:

- Wastewater
- Sewage sludge
- Agricultural land contaminated by fertilizer residue
- Sediments
- Brownfield and other heavy metal contaminated soils

In addition a considerable amount of work has been conducted to examine the phytoremediation potential of *Salix* in glasshouse conditions using hydroponic studies.

Wastewater

The use of *Salix* for phytoremediation began in the late 1980's and early 1990's as a by product of research into increasing yields of these trees using nutrient rich sewage sludges and waste waters that were also high in heavy metals. Perttu (1993) suggested SRC could be used as vegetation filters, utilizing nutrients in sewage sludge and wastewater when applied to SRC plantations. The increased nutrient demands of some fast growing willow taxa proved useful in removing high nutrients concentrations found in wastewaters. Based on a high water demand in the root system this would lower nutrient leaching into the surrounding environment (Sander and Ericsson 1998). Experiments conducted in a hydroponic system screened 13 *Salix* taxa for nutrient uptake efficiency, concluding that *Salix viminalis* 'Gigantia' would be most effective for effluent remediation due to high N and P uptake and high biomass productivity (Alker *et al.* 1997). The high water demand of many SRC species may also allow an element of hydraulic control, for example to control a polluted groundwater plume.

Sewage Sludge

The utilisation of sewage sludge and other contaminated waters revealed that certain taxa of *Salix* possessed the ability to concentrate heavy metals within their biomass. The use of sewage sludge was proposed as a replacement for costly artificial fertilizers (Landberg and Greger 1994) and the concept of using the sludge as a fertilizer to improve commercial biomass whilst the same time charging for waste disposal was recently investigated (Bardos *et al.* 1999).

Riddell-Black (1994) investigated four *Salix* taxa planted on land that had received continued application of sewage sludge for 50 years, with heavy metal concentrations elevated above those normally permitted. Although all taxa accumulated metals to varying degrees within the above ground biomass, it was calculated that 126 years would be needed for reduction to target levels for the highest concentrated element, Cadmium. A similar study was expanded to examine twenty *Salix* taxa, planted on land with elevated metals from sewage sludge application (Riddell-Black *et al.* 1997). This study showed considerable variation in uptake by different taxa, suggesting it may be possible to screen for accumulating and non-accumulating taxa to satisfy differing remediation requirements, such as stabilization or extraction. The study also concluded that uptake of Cd and Zn did not limit yield, whereas yield was limited in taxa that were able to accumulate Cu and Ni. Further analysis of this experiment published by Pulford *et al.* (2002), stressed the importance of considering biomass yield alongside metal uptake characteristics if ultimately attempting a reduction in soil metal concentrations. The study also considered the effect trees could exert on metal mobility in the surrounding soil, showing that soil EDTA-extractable Cd, Cu, Ni and Zn increased under areas planted with trees compared to those left unplanted. Another conclusion from the study reinforced statements previously made by

Ernst (1996), that this form of remediation may be suitable for the 'polishing' of sites that were only slightly contaminated.

Agricultural / Fertiliser

High concentrations of contaminants such as Cadmium are frequently found in agricultural land where sustained use of fertilizers has introduced Cd to the soil as a by product. Farmland across Sweden contains elevated concentrations of Cd in the topsoil (Ostman 1994). It is undecided if the ability of *Salix* to accumulate Cd is beneficial as a remediation tool, or problematic when trying to produce a biomass crop (Perttu 2002). Other authors take a different view, claiming that *Salix* can be used effectively as a remediation tool for cadmium in order to prevent Cd transfer into other arable crops that may be for human consumption (Berndes *et al.* 2004).

Sediments

Planting willow on contaminated sediments and dredgings has been investigated for the remediation of organic and inorganic contaminants. Sediments can retain a variety of different contaminants that may not degrade under reduced conditions. Willows are able to exploit waterlogged ground conditions and planting is possible on difficult substrates using the SALIMAT system (Vervaeke *et al.* 2001). *Salix* planted in this way on a dredged river sediment were able to effect a 57% reduction in mineral oil and accumulated Cd and Zn in their biomass (Vervaeke *et al.* 2003), calculating that it would take 33 years or 11 successive rotations to reduce Cd concentrations to 2 mg kg^{-1}, the Flemish standard for use of dredged sediment as soil. Of considerable interest, was the use of *Salix* as a stabilization tool, to ensure an element of physical stability and reduction of leaching through hydraulic control. Vandecasteele *et al.* (2002) observed *Salix* taxa that had naturally colonized sediment landfills, not those bred for biomass purposes, and suggested a possible threat from habitat development of trees that accumulated quantities of Cd and Zn due to trophic transfer.

Brownfield Land

Relatively few studies have been made in comparison of the use of willow to remediate other types of land, particularly soils with relatively low levels of contamination from a variety of anthropogenic sources and distributions. Many studies on brownfield land to date have used amendments such as sewage sludge, or have been based on mine spoil and other similar sites.

Hydroponics

Many field and pot studies were preceded by observing the uptake potential of willow in hydroponic culture. This technique has been used extensively to examine uptake rates and tolerance indices in a controlled environment. Increased shoot metal concentrations are found when plants are grown in hydroponics compared to field grown plants, but it appears that clones with higher rates of metal transfer to shoots can be readily identified using hydroponics (Greger and Landberg 1999; Pulford and Watson 2003). It has recently been used as relatively quick and effective screening tool for the potential use of *Salix* taxa in the environment (Watson *et al.* 2003). This study also examined the comparison between uptake data achieved in hydroponic conditions and that achieved for the same taxa grown in the field.

Salix has also been investigated for radionucleide uptake, with specific aims of changing land uses to biomass production after localized radioactive contamination, such as Caesium (Gommers *et al.* 2000; Goor *et al.* 2001). In the case of the former experiment radionucleide transfer into the wood was low, indicating the potential use as a crop for radionucleide impacted farmlands where the growth of other plants for human consumption may no longer be safe.

1.10 Poplars

The use of poplars in remediation follow a similar pattern to *Salix* and have shown an affinity for nutrient immobilization (Berthelot *et al.* 2000). *Populus* has received considerable attention in the phytoremediation of organic contaminants (Burken and Schnoor 1998). Hybrid poplars have been used to remediate organic contaminants such as High Explosives, (Yoon *et al.* 2002); TCE, (Newman *et al.* 1997; Shang and Gordon 2002); Petrochemicals, (Rentz *et al.* 2003) and Atrazine (Burken and Schnoor 1997). Much less consideration however has been given to their use to remediate heavy metal contaminated soil.

Moffat *et al.* (2001) tested several *Populus* taxa, on treatments of sewage sludge with and without irrigation, resulting in average stem concentrations of Cd, 6.6, Zn, 51, Cu, 5.5 (all values mg kg^{-1}). Foliar data was not presented but Zn and Cu were significantly elevated. Sebastiani *et al.* (2004) conducted pot trials of two *Populus* taxa amended with organic industrial waste with elevated Zn and Cr. After one year Zn concentrations reached 300 mg kg^{-1} in foliage, 80mg kg^{-1} in stem and 100 mg kg^{-1} in roots. Cu and Cr were not significantly elevated, although Cu uptake into roots was 5-6 times higher than maximum stem uptake. Di Baccio *et al.* (2003) conducted similar experiments in pots spiked with a Zn solution. At the maximum Zn concentrations (1000μM), leaves accumulated 160 mg kg^{-1}, stem 60 mg kg^{-1} and roots 90 mg kg^{-1}. Other studies have shown it is possible for Poplar to accumulate Cd (Robinson *et al.* 2000), but this involved the use of chelating agents to improve Cd availability to poplars and had undesirable side effects such as growth reduction and leaf abscission.

Poplar has received recent attention in the UK in a silvercultural context. New high yielding Belgian hybrids have been grown successfully in the UK with good commercial yields. This has been coupled with the renewed interest in traditional lowland forestry and UK agricultural land reform (Tabbush and Beaton 1998; Tabbush and Lonsdale 1999).

1.10.1 Phytoremediation properties of other tree species

Other tree species have been planted on brownfield or contaminated land because of physiological factors that make them suitable for planting on hostile substrates; such as deep rooting ability, drought tolerance or metal resistance. *Betula pendula* is well documented to be able to tolerate increased levels of heavy metals, and is a hardy pioneer species (Utriainen *et al.* 1997; Kozlov *et al.* 2000). Goransson and Philippot (1994) examined the use of *Betula pendula* as a 'metal collector' in relation to cadmium uptake and found almost 100% of Cd added via amendments was subsequently found in tree seedlings.

Alnus incana is a popular choice in forestry based restoration due to its nitrogen fixing properties and ability to survive on poor quality soils (Moffat and McNeill 1992; Moffat 2000). Loblolly Pine (*Pinus taeda*) seedlings were grown in sewage sludge by Berry (1985) to evaluate the usefulness of sewage sludge as a nutrient source, indicating that ectomycorrhizal production was reduced and metal uptake into the trees was not significant.

Eucalyptus (Pyatt 2001; Bhati and Singh 2003) and *Miscanthus* (Wilkins 1997) are other species that have also been examined for their phytoremediation potential, but are not discussed further in this study.

1.11 Disposal Technologies

If phytoextraction is to be a successful remediation option then careful consideration must be given to the processing and ultimate disposal of plant material that may contain high levels of heavy metals. This critical step in successful remediation is frequently overlooked. Garbisu and Alkorta (2001), Mulligan *et al.* (2001) and Sas-Nowosielska *et al.* (2004) present a review of specific technologies and examine the current technology status. This topic is discussed in more detail in Chapter 7.

1.12 Planting trees on derelict land

Forestry is a valuable tool for reclaiming large areas of landscape obliterated by industrial or other destructive processes (Perry and Handley 2000). The advent of community forestry and urban greening to reclaim brownfield sites has reinforced this concept. The Mersey Forest realised that to meet its ambitious planting targets it must increase planting on derelict land (Mersey Forest 1999b). Recently soft end development of brownfield sites has been the preferred option of regional development agencies keen to encourage low cost and high social value schemes. The Newlands project in the North West will spend £23 million pounds redeveloping 435ha of derelict and brownfield land using forestry (NWDA 2003).

However, tree establishment can encounter many obstacles resulting from the nature of the land used for planting, which can apply equally to amenity restoration, biomass forestry or phytoremediation schemes. The nature of brownfield sites is implicitly varied from ex-agricultural land to highly toxic bare mining wastes, but the same minimum soil requirements are necessary for trees to survive and establish.

Moffat and McNeil (1994) produced the first practical integrated guidance, based on research carried out in the previous 30 years, for establishing trees on derelict land. Later guidance and research was aimed at specific land types such as landfill (Dobson and Moffat 1993, 1995; Mersey Forest 1999b), former mineworkings and colliery sites (Bradshaw 1997; Bending and Moffat 1999), and contaminated land, (Dickinson 2000; Dickinson *et al.* 2000; Pulford and Watson 2003) although there is considerable overlap in many of these examples. The most recent tool for assessing if tree planting is viable on a range of land types is presented in a software package and decision support tool, Roots (Forestry Commission 2003). Guidance now calls for a careful appraisal of a number of site factors before tree planting can be sanctioned. These considerations will not only apply to soils already present on a site, but also frequently to soil forming materials where it is necessary to create an organic substrate where one may have not existed previously. These materials may already be present on derelict sites in the form of spoil or overburden, in some cases they may represent the majority of the soil material (Bending *et al.* 1999).

Common criteria to consider when establishing trees on brownfield land are:

- Soil Bulk Density and Permeability
- Soil Depth
- Cultivation and Placement
- pH
- Electrical Conductivity
- Soil Pyrite Content
- Contaminants (inc organic, inorganic, wastes, methane, CO_2 etc.)

Soil contamination is only one issue in a variety of factors that will affect tree planting. Soil compaction is a serious problem on brownfield sites, resulting in poor tree rooting, stability and nutrient supply (Hutchings *et al.* 2001) and the depth of soil used for planting will have a profound effect on tree survival (Moffatt 1995). There is a strong driver to use forestry as a soft end use for contaminated sites as it requires less stringent clean up goals although must be conducted in a predictive risk based framework (Hutchings 2002).

1.13 The use of forestry as a soft end use for land reclamation – a multi functional approach

The use of forestry to reclaim derelict and contaminated land has many environmental, social and economic benefits. It is often the case that communities blighted by dereliction are trapped in spiral of decline. Ward (1996) commented, "successful regeneration of whole areas... physical and psychological, can depend on improvements to the local environment". Global trends and changes in economic land use have left large quantities of contaminated land in close proximity to people, and frequently in areas that already suffer a degree of social problems and deprivation (Barton 2000).

Ward (1996) also suggested three broad approaches for using trees on disturbed land. The Forestry approach; driven by economics, slow results and easy management, the landscape approach; not commercially viable and requiring more intervention and the ecological approach; low cost, low input, natural processes. Perry and Handley (2000) expanded this concept to include the important factors of recreation, in the form of community forests and improving local environments either though improving atmospheric conditions (Freer-Smith *et al.* 1997.), or though overall soil improvement by the action of growing trees. The National Urban Forestry Unit had suggested many of these approaches in 1992, but it would take time for the wider reclamation community to accept it was actually possible. It may now be possible to add an element of risk management as a further approach to planting trees on derelict land.

New schemes are being advocated to use Short Rotation Coppice (SRC) not only for environmental regeneration, through phytoremediation, but also for social regeneration. Paulson *et al.* (2003) discusses the considerable benefits of SRC remediation schemes to the intermediate labour market, delivering sustainable outcomes and outputs such as local employment, neighborhood renewal, community health and a recreational or leisure resource.

Tree planting for economic return is a core industry. Steer (1997) conducted experiments comparing the yields of poplar, willow and alder grown on ex-agricultural land to those grown on reclaimed coal tips. Organic amendments were used on the former coal tips to improve organic matter content and increase tree growth. Bardos *et al.* (1999) and exSite Research (2001) continued the theme of productive re-use of derelict land, but suggested that importing sewage sludge as an amendment could also be used to generate revenue through a waste management fee, as well as helping to produce a coppice product (Figure 1.1). The environmental and social importance of this system was noted.

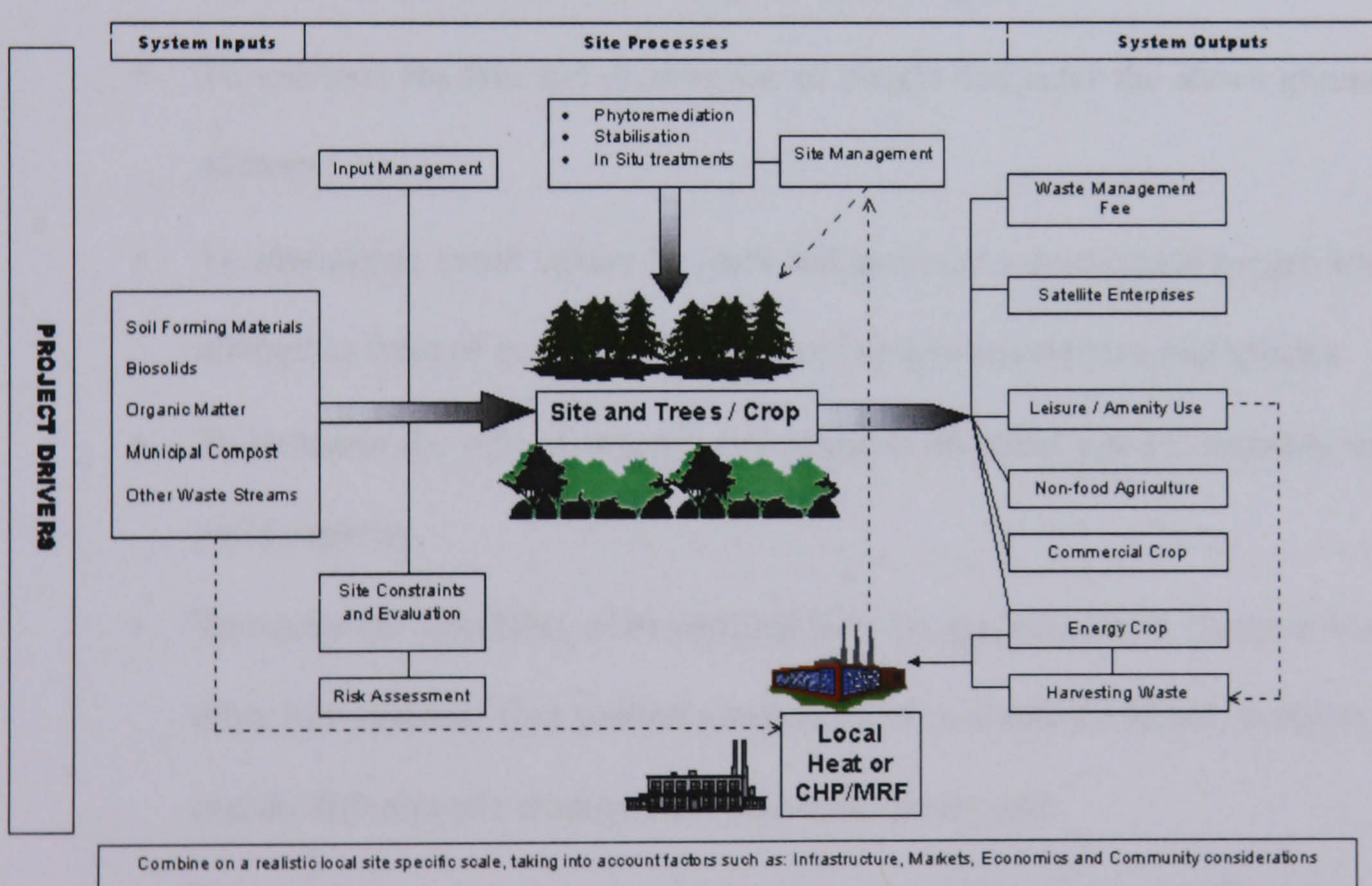


Figure 1.1. Intergrated system for SRC production and fee income from waste management and crop sales (originally published in Bardos et al. (1999); exSite Research (2001)

1.14 Aims and Objectives

The aims of the research reported in the present study were:

- To establish and maintain a network of experimental plots at field sites contaminated with a range of heavy metals, growing a combination of *Salix, Populus, Larix, Betula* and *Alnus* tree species.
- To investigate and develop a methodology for mapping metal contamination, including assessment of differing chemical extraction techniques.
- To examine how different guidance regimes may affect potential phytoremediation schemes and the treatment of contaminated and brownfield land.
- To investigate the performance of tree species in phytoremediation and the mobility of metal contaminants within the plant and soil systems.
- To examine the fate and distribution of metals that enter the above ground portions of trees.
- To investigate metal uptake by trees and potential toxicological trigger levels and to attempt to form of generalised patterns of uptake among taxa and species.
- To examine the role of organic amendments on metal uptake, mobility and overall yield capacity.
- To assess the suitability of brownfield land for growing Short Rotation Coppice and other tree species. Can general predictions of potential yield and mortality be made, and do different site management practices affect yield.
- To investigate potential treatment rates and reductions in metal burden of the soil and associated methods of disposing of contaminated biomass.

Chapter 2: Site Descriptions

Sites for full-scale experimental fieldwork were selected in collaboration with The Mersey Forest and other project partners, including local authorities and private industries. Sites were short-listed to reflect different historic and current land uses, type and concentration of contaminants and geographical location. Some sites were initially selected for investigation then withdrawn due to access issues or lack of contamination. The project team suggested additional sites to broaden the range available and overlap with previous research work at both Liverpool John Moores University (JMU) and University of Liverpool (UoL).

2.1 Sites of Study

Eight separate field sites were selected for experimental work, but this number was reduced to six after two sites became unusable due to site access difficulties.

This chapter considers each site in turn, briefly examining its location, history, contamination factors and reasons for being included in the project. The broad context of each site is described in the present chapter, whereas details of experimental plots siting, treatment and detailed chemical analytical work are discussed in later chapters. Of the six selected sites, four had been the subject of a phase one site investigation conducted on behalf of The Mersey Forest Brownfield Project in 1999. Information from these investigations was made available to the project team and has been used as the basis for some of these descriptions. In many cases, historical maps and aerial photos have been used to provide information on previous land uses and site history. This information is of considerable use in the context of assessing contaminated sites (BSI 2001) and planning long term strategies for 'fit for use' development.

Table 2.1 shows an overview of the sites; henceforth sites may be referred to by their full name or site code abbreviation.

Table 2.1. Summary of sites used for experimental trials

Site	Site Code	NGR	Owner / manager	Phase 1 report
Sugar Brook	**SUG**	SJ 392960	Liverpool C.C.	Yes (1999)
Fazakerley Reed Bed Scheme	**FAZ**	SJ 387963	Private / NWW	No
Kirby Moss	**KIR**	SJ 445984	Private	Yes (1996)
Merton Bank	**MER**	SJ 527960	St. Helens MBC	Yes (1999)
Deeside Steelworks (Corus)	**COR**	SJ 308703	Private	No
Cromdale Grove	**CRM**	SJ 534947	St. Helens MBC	Yes (1999)

Figure 2.1. Geographical locations of experimental field sites within the Merseyside region.

In the following sections, aerial pictures for each site show site boundaries marked in red, and experimental planting plots marked in black.

2.2 Sugar Brook

2.2.1 Overview

Sugar Brook is 3.8 ha of former allotment land situated in the Fazakerley area of Liverpool, a dense urban area of major arterial roads, housing and mainly defunct industry. The land has had no designated use for at least 20 years. Existing vegetation consists of mainly neglected, irregularly mown grasslands and dense willow and bramble scrub.

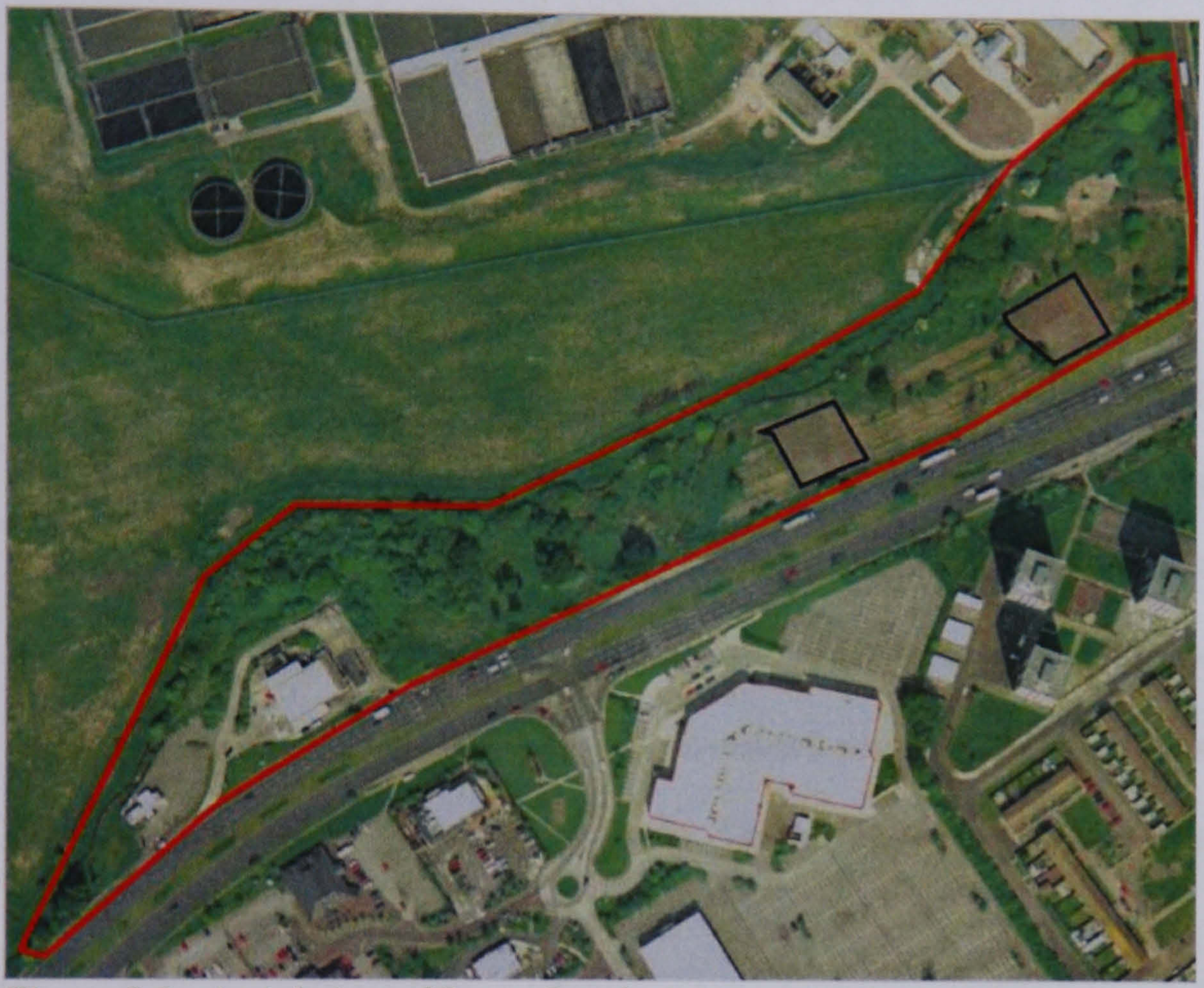


Figure 2.2. Aerial view of Sugar Brook site

2.2.2 Site Details

The site is 3.8 ha in area and is distributed in a linear orientation alongside the main A580 trunk road connecting Liverpool to Manchester, less then 2km from the Liverpool City Boundary. The site is adjacent to a BP petrol filling station, the area to the East being owned by Liverpool City Council and the main focus for this study, as areas to the West were difficult to access. A large sewage treatment works to the North and the main A580 road to the South act as a site boundary. Beyond the road are mixed retail units, a cinema and several tower housing blocks. Residential areas of North Liverpool are to the west and an industrial corridor follows the A580 to the east. Within the site, Sugar Brook flows as a 1.5m deep channel along the northern edge, flowing into the River Alt outside the site boundaries.

Several road drainage channels cross the site flowing into the brook; these are of poor water quality.

The site is not currently under any formal management, other than occasional dredging of the Sugar Brook channel by the Environment Agency. Although the site is located in the middle of a dense urban area, with the exception of fly tipping and petty vandalism in the form of fires, there is no formal public access to the site.

2.2.3 Site history and brownfield status

In 1897 the site was under agricultural production. Sugar Brook ran in its present position to the north of the site, and the fields beyond the brook were marked as a sewage farm. The surrounding area was agricultural. The present day shape of the site had developed by 1938, but still within a largely agricultural landscape. The road had been constructed along the southern boundary and the sewage works extended considerably to the north to include large structures such as settling tanks and beds. Beyond this, to the north, south and west there was evidence of residential developments.

Residential developments continued to grow through the 1950's and 60's. On the 1967 map the site was labeled as allotment gardens and the road had become a dual carriageway. The eastern part of the site had become a small development, possibly an extension to the ever-expanding sewage works. Engineering and electrical works are marked to the east of the site. By 1974 the development on the eastern part of the site had disappeared. Only the western part of the site remained marked as allotments. The number of structures at the sewage works had increased and residential developments to the south and east had increased also. The garage appears in the mid 1970's bisecting the site

In the late 1980's the site was no longer labeled as allotment gardens and the map showed just mature trees. The works to the east had been vastly reduced in size, leaving open, derelict ground. Residential development had increased in the vicinity of the site.

2.2.4 Contamination at Sugar Brook and reasons for selection of the site

Over a period of more than 100 years the site remained largely under either agricultural, allotment or public open space use. There is no direct evidence of any historical contaminative processes taking place on the site, and the Environment Agency reports no records of contamination or recent pollution incidents arising at the site. The petrol station in the middle of the site provides a potential direct source of contamination from spillage or underground storage tanks, although no evidence has been found to date. The site has a number of indirect pollution sources which are discussed below.

As the site history clearly shows Sugar Brook does not have any record of particular contaminating practices on the site, despite being previously identified as a site with contamination issues by the local authority. Of particular interest however is that Sugar Brook typifies a great many derelict sites in Merseyside. These are relativity small sites that have been neglected for a number of years, effectively partitioned by development on all sides and now situated in a dense urban or industrial context. Sugar Brook's proximity to industry, treatment works and major roads make it undesirable for residential development, reinforced by local issues of dereliction and blight. The location of the site means that it has been subjected to several indirect pollution vectors, such as road runoff, aerial deposition, fly tipping, agricultural chemicals, poor quality waters flowing though it and the potential for sewage sludge from the adjacent works having been dumped on parts of the site in the past. The poor economy of the area and large availability of previously developed and derelict land meant that it was unlikely to be developed for economic or industrial use in the foreseeable future. A soft end use, featuring community forestry, may be desirable for ecological, landscape and economic reasons.

2.3 Cromdale Grove

2.3.1 Overview

Cromdale Grove is 8.8 ha of predominantly mown amenity grassland situated in the Parr area of St. Helens, Merseyside. Residential areas and industry surround the site. The site was previously known to have been a landfill, receiving domestic, industrial and chemical waste. In its present day form it is used extensively for informal recreation and dog walking.

Figure 2.3. Aerial view of Cromdale Grove

2.3.2 Site Details

The site is comprised of 8.8 ha in one large, flat, almost square area of amenity grassland. It is surrounded by large housing estates to the north and east; properties to the north are situated directly adjacent to the site boundary and a secondary school lies to the south and south east. To the west, the Parr Industrial Estate borders the site and Sutton Brook flows though a very deep channel on the south of the site and delineates the southern boundary. Beyond the brook to the south lie more industrial areas. The majority of the site is mown amenity grassland, except for some pockets of scrub willow and planted woodland on the western fringes; this woodland is unmanaged and in a poor state. Towards the southern boundary and Sutton Brook the land is frequently waterlogged with associated marsh plant species.

Soil depth is generally shallow, with only 10-30cm of soil present in many places, below which lies landfill waste. The site is popular with dog walkers and is also used for other forms of informal recreation. The site is linked with other areas of open space within the St. Helens area via the Sutton Greenway project and provides a significant amenity resource within a densely populated area. However, the site is also subjected to problems of fly tipping in the woodland to the west, motorcycle riding and frequent bonfires.

2.3.3 Site history and brownfield status

In 1846, the site was within a predominantly agricultural landscape, although mine shafts were evident to the north and a chemical works was situated on the SW corner of the site. The site is bounded by Sutton Brook to the south. By the late 1890's the number of industrial sites in the vicinity had increased rapidly, especially to the south of the brook with a mill and tile and glass works. A colliery existed to the NW, with a railway to the west. The chemical works to the SW were now labeled as Alkali works. The site itself remained as fields. In the early 1900's brick and tile works had appeared to the West of the site. By 1938 the colliery to the NW was no longer marked, although the spoil heaps remained; beyond this was evidence of residential development. The site was still comprised of fields, although depressions that were probably ponds had increased.

By 1965 the number of residential properties surrounding the site had increased to the north and east, and the industrial site to the west was also expanding. This continued though the 1970's, with the addition of the school in the SE with part of the site being marked as playing fields. The railway to the west had been dismantled by 1974. Aerial photos from the 60's and 70's showed different areas present on the site, including depressions and ridges, most likely associated with landfilling.

2.3.4 Contamination at Cromdale Grove and reasons for selection of the site

The continual presence of many primary industries surrounding the site since the 1850's suggests there may be a significant level of pollution associated with the site. The alkali works adjacent to the site in the latter half of the 19th century could have been expected to release some waste materials onto the site, whether deliberately or accidentally. Other surrounding industries such as coal mining, milling and ore processing were all associated with unregulated dumping of materials throughout their operation. Evidence from aerial photos and St Helens MBC suggests that deliberate filling of the site occurred during the 60's, either as a disposal route or in an attempt to level the area. It is documented that filling may have also occurred previously.

Several trial pit surveys have been conducted on the site to try and quantify the underlying fill. The latest, in 1997 concluded the site was filled with colliery spoil, ash, clinker, rubble, alkali waste and household waste. These are deposited heterogeneously throughout the site in area and depth and often associated with areas of heavy metals such as copper, nickel and zinc.

Given the surrounding industry, especially the colliery, the site may have received leachate from other off site sources that may have contributed to pollution problems. Although domestic waste was filled in the 1960's its proportion of the total waste filled appears to be quite small. Due to the amount of time it has been in the ground, any methane production is likely to be minimal. Leachate from the site has been observed on occasions entering the adjacent Sutton Brook, thus adding an element of off-site contamination migration.

The concentrations of heavy metals such as copper and zinc, and to a lesser extent nickel, were sufficiently elevated above existing guidance in places. The distribution of these metals is extremely heterogeneous as would be expected of an area receiving many differing waste types over a long period of time. Due to these elevated concentrations and the perceived constraint to growing woodland, several applications to plant trees on the site have been denied by statutory bodies such as the Forestry Authority. The site itself is a locally important area for recreation and wildlife and as an area of green space within a dense urban setting. For this reason the site requires some development of habitats and public accessibility whilst ensuring that such schemes are successful and potential exposure to potentially harmful elements is minimised.

2.4 Fazakerley Reed Bed Scheme

2.4.1 Overview

This scheme occupies land owed by North West Water and was previously part of the nearby sewage treatment works. The site is very close to Sugar Brook, which is located 250m to the south. In 1998 the site was redeveloped to allow a previously culverted stream to flow above ground though a reed bed treatment system to improve the poor water quality of the area.

Figure 2.4. Aerial view of Fazakerley Reed Bed site

2.4.2 Background

Urban development has resulted in many natural drainage channels, brooks and streams being culverted to facilitate industrial and residential development. In North Liverpool, Tue Brook was culverted for almost its entire length, receiving a mixture of industrial runoff and contaminated urban drainage from a variety of diffuse and therefore largely untraceable sources along its length. The quality of the water entering Fazakerley Brook and in turn the River Alt was very poor, frequently containing high levels of copper and other contaminants (WS Atkins and Environment Agency 1998) This effect is exacerbated by the culverted nature of the stream as opportunities for deposition, oxygenation and interaction with vegetation were removed. At this site it was assumed that by re-routing the culvert above ground and introducing a wetland treatment facility, water quality could be improved before discharge to the River Alt.

2.4.3 Site Details

The site consists of semi-natural grassland vegetation, including mixed rudural herbs and natural grassland. Recent redevelopment of the site provided extensive planting of trees during 2000-2001. The site lies in close proximity to Sugar Brook and shares many similarities in its surrounding urban environment. Large residential areas lie directly to the north with the sewage treatment works bordering the site for its entire eastern boundary. Recreation fields and Fazakerley hospital are situated to the west and Sugar Brook to the south.

The re-engineering of the site has created an extensive new water channel that now carries the previously culverted Tue Brook, above ground through the entire length of the site. The channel has been further engineered to include two large settling pools and a reed bed treatment area, before discharging into Fazakerley Brook at the northern edge of the site.

During land forming operations all existing vegetation was lost or significantly reduced. Natural recolonisation has been rapid with large communities of ruderal grasses and forbs dominating the site. Several large areas were planted with a mixture of broadleaf and conifer tree species, and a new hedgerow was planted along the western edge of the site. Soil depth is greater than 1m, and a rapid establishment of invasive vegetation indicates a high level of fertility. The site is currently under no specific management regime, although Lancashire Wildlife Trust were due to take over site management. Some gradual expansion of the tree-planting programme is planned and the site remains restricted to the general public.

2.4.4 Site history and brownfield status

The site has been part of a sewage farm since 1897, although seems to have been separated from the main part of the sewage farm site by a boundary marker since 1908. No development has taken place on the site and whereas the sewage farm to the east has been expanding with filter beds and other infrastructure, this site has remained unchanged. The only exception is the addition of several roads linking the site to the main part of the sewage works which appear in the 1960's, although these were removed by 1988.

2.4.5 Contamination at Fazakerley Reed Bed Scheme and reasons for selection of the site

Evidence of any contamination process occurring directly on the site is limited. As the site was originally a sewage farm and has remained part of a sewage treatment works for over 100 years, it would be reasonable to assume that some transfer of sludge to land may have occurred over this time. Dumping sludge to land was a known disposal route and there is some anecdotal evidence this occurred across the site. However, considerable landscaping earthworks have been performed on the site and any contamination that may have been present in the top layers of soil may now be buried beneath the surface.

The consulting engineers, WS Atkins, conducted a limited amount of soil testing for metals prior to the re-engineering scheme commencing. Nine sampling locations were assessed from across the site with the following maximum concentrations recorded (all samples taken from between 0 and 1m below ground level): As 87mg/kg, Cd 5.0 mg/kg, Cu 402 mg/kg, Ni 57 mg/kg, Pb 556 mg/kg and Zn 360 mg/kg (WS Atkins and Environment Agency 1998). The metal concentrations are elevated above background levels and confirm that sewage sludge disposal may have occurred on the site historically.

Any contamination flowing through Tue Brook is unlikely to impact directly on the land as the channel has been clay lined, although any flash flooding events may circumvent this.

Potential problems may also arise in the future as contaminated sediment drops out of solution and deposits in the stream bed and settling pools. Sewage sludge consistently contains high concentrations of heavy metals and, although recent legislation has tightened up amounts permissible in sewage waste (MAFF 1993), this site may have been receiving sludge for over 100 years, long before these guidelines were conceived.

This site presented opportunities to manipulate soil conditions. The possibility of using additional organic amendments such as sewage sludge and sewage cake is possible due to the lack of public access, as is the possibility of irrigating experiments with contaminated water as it enters the site.

2.5 Merton Bank

2.5.1 Overview

Merton Bank is an area of public open space (6.6 ha) in the Pocket Nook area of St. Helens, Merseyside. It consists predominantly of intensively mown amenity grassland interspersed with small patches of wayside vegetation and planted woodland. The site is in the middle of a dense residential and industrial area and is well used for public recreation. The site was known to have received a wide variety of industrial waste although much of its composition is unknown.

Figure 2.5. Aerial view of Merton Bank site

2.5.2 Site Details

The site comprises 6.6 ha of predominantly mown amenity grassland, except for some pockets of planted trees. The margins of the site are unmown and these areas are reverting naturally to wayside plant communities. The topography consists of several ridges and mounds giving an interesting raised landform in an otherwise largely flat landscape. The St. Helens Canal defines the northern boundary, and mixed residential properties are found to the east and northwest. To the South lie school playing fields and a nursing home. The west of the site consists of expansive industrial estates.

Soil depths are generally low, with only 10-30cm of soil present in many places, below this waste materials are frequently encountered. The site is managed by periodic mowing of the grassland. It is popular with dog walkers and other forms of informal recreation. The site is linked with other areas of open space within the St. Helens area via the Sutton Greenway project and provides a significant amenity resource within a densely populated area. However, the site is also subjected to problems of motorcycle riding, sporadic bonfires and other anti-social behavior.

2.5.3 Site history and brownfield status

In 1849 the entire site was used as a reservoir to feed nearby mills. The land was subsequently drained and by 1873 an alkali works had been constructed on the eastern part of the site for the manufacture of sodium carbonate using the Leblanc process, although the works were removed 20 years later. The St. Helens canal ran to the north of the site, with farmland to the east and south and the start of residential development to the west. Many industries were situated close to the site including oil and tallow works, refineries, alkali and chemical works. From the 1900's, the site existed as a raised piece of land in a heavily industrialized landscape, and aside from the addition of a railway line on the south boundary of the site and an increase in the number of surrounding chemical industries the site remained relatively unchanged for many decades.

By 1965 a decrease in the number of surrounding industries could be seen. Residential developments to the north, east and south were clearly visible and a new road bordered the west of the site. The ditches and brooks that crossed the site had all been filled in sometime after 1956. During the mid 1970's the railway line had become disused, although the site was designated as a public amenity area in 1977. The reclamation of two large conical domes of alkali waste commenced in 1978 with landforming to lower the waste tips at each end of the site, and raise the central area. Once complete a 23cm layer of colliery spoil from the nearby Clockface Colliery was added and left for 3 months before two ripping operations to open the substrate to wintering and facilitate mixing. Areas of tree planting also received sewage sludge and additional substrate mixing (Murphy 1979).

Residential development to the north and east continued throughout the 1980's and 90's and to the west new industrial units have been erected along the boundary road. The site remains as undulating land varying in height from 30 to 35m AOD. By 1992 relatively mature trees were present on some parts the site.

2.5.4 Contamination at Merton Bank and reasons for selection of the site

Apart from the alkali works that were demolished before the turn of the century, no other contaminative processes have been situated directly on Merton Bank. However, the site has remained within a densely populated area of chemical, mining and refining industries for over 100 years. A report on the site conducted in 1979 states, "after production ceased (approx 1890's) the site consisted of two large conical tips of alkali waste with a central area of rubble.... and has remained untreated for over 70 years" (Murphy 1979).

Chemical testing from a site survey in 1999 (Mersey Forest 1999a) revealed extremely high concentrations of arsenic (As) distributed heterogeneously across the site. The present study shows that Merton Bank contains extremely high levels of arsenic, and several other heavy metal contaminants, which are discussed at length in Chapter 4. The soil dynamics and plant uptake of arsenic is still relatively poorly understood in the context of phytoremediation, especially in relation to trees (Mattusch *et al.* 2000; Zhang *et al.* 2002).

The site shares many similarities with Cromdale Grove from a community viewpoint; it is a very important area of amenity greenspace in a highly developed area, but may benefit from improved landscape areas, including community forestry. The potential exposure of the public to contaminated material must be addressed under new CLEA legislation (DEFRA and Environment Agency 2002a) and targeted remediation methods such as phytoremediation may play a part of this.

2.6 Kirby Moss

2.6.1 Overview

The Kirby Moss former landfill occupies an area of approximately 34 ha and is a privately owned site surrounded by agricultural and grazing land. The landfill closed in the 1970's and the land has been used for periodic agricultural grazing since. This site was used as part of the Mersey Forest Landfill Project and contains 0.66 ha of tree planting from 1998.

Figure 2.6. Aerial view of Kirby Moss

2.6.2 Site Details

The site is divided into a western phase known as Coopers Moss and an eastern phase, Kirby Moss, only the eastern phase was utilised for this project. A large, flat, raised field forms the site that has been used to graze cattle. The western boundary is formed by the Perimeter Road. A drainage ditch, contaminated with leachate, forms the southern boundary.

A cattle grazed grassland sward consisting of mainly, *Arrhenatherum elatius, Dactylus glomerata, Holcus lanatus* and *Lolium perenne* dominates the vegetation. Additionally, an area of diverse flora has developed within the previous tree planting experimental exclosure. Ground disturbance during cultivation and a reduction of competitive weeds has promoted locally rare orchid species. Some mixed scrub vegetation also occurs towards the southern boundary of the site. Soil coverage is generally poor. Topsoil and rootable material depths are 0.15 m and 0.6 m respectively. In some areas no topsoil was recorded and there was evidence of waste materials brought to the surface during ground preparation.

2.6.3 Site history and brownfield status

Detailed historical site information was not available for this site, although it was probably a wetland area (a moss) that was used as a landfill site after peat extraction. The site ceased landfill operations in 1970. Domestic, construction, commercial and industrial materials were accepted at the site to an unknown depth.

In 1998, 0.66 ha of experimental tree planting was added and managed as part of a research project by Liverpool John Moores University, The University of Liverpool and The Mersey Forest into tree planting on closed landfill sites (Putwain *et al.* 2003).

2.6.4 Contamination at Kirby Moss and reasons for selection of the site

Previous studies conducted on the site indicate that phytotoxic contamination is present in the soil. Thirty percent of soil samples taken from between 0-0.5 m below ground level were contaminated with Cu, Zn or Ni in excess of the ICRCL threshold for phytotoxicity (Rawlinson 2001). Below 0.5 m the proportion of contamination increased to 46%. The soil chemistry was described in the preliminary site investigations, as 'aggressive' due to 'phytotoxicity and locally acidic conditions (Allot and Lomax 1997)

A substantial area of Kirby Moss is affected by landfill gas. At 0.75 m almost the entire horizon was affected by carbon dioxide (1-10 %). Methane concentrations of 5-15 % were present, at 0.75 m, along the western boundary of Kirby Moss extending eastwards through the centre of the site. Previous reports have suggested landfill gas would appear to pose a significant threat to the long-term viability of the woodland stand within the experimental area (Rawlinson 2001).

2.7 Deeside Steel Works (Corus)

2.7.1 Overview

The Corus steelworks are situated adjacent to the Dee Estuary in Flintshire, North Wales. This expansive site has been in used as a steel manufacturing facility for over 100 years although recently steel production has ceased and been replaced by a specialist metal coatings facility.

Figure 2.7. Location map of Deeside steel works site

2.7.2 Site Details

The large complex contains a network of road, rail and building infrastructure consistent with a large primary industrial site. General site details are neither necessary nor available and specific information associated with experimental planting sites is discussed in Chapter 4.

2.7.3 Site History and Contamination Issues

Steel works have been present on the site for over 100 years. No specific details on previous land use or contamination incidents are available. It is known that sump oil leakage from an adjacent steel mill has drained onto part of the site over several decades. This is still in evidence in drainage ditches and culverts across the site. The area for experimental planting did receive hydrocarbon contaminated dredging in 1996 as part of a trial into land farming to improve soil conditions for possible bioremediation.

2.7.4 Relevance to the Project

The area to be used for the experiment contains large quantities of mineral oil and potentially other organic by products, offering an opportunity to examine the role of trees in phytoremediation of organic contaminants, along with any inorganic contaminants likely to be present within the oil.

Chapter 3: Materials and Methods

3.1 Site Selection

Sites for experimental work were selected according to a range of criteria outlined in Chapter 2, which also explains the background of the sites.

3.2 Plot Selection

Plots for experimental tree planting were selected on the basis of attempting to situate them over or around contaminated areas of the site. Where data were available from previous Phase 1 site investigations they were used to produce visual indications of site-wide contaminant concentrations. This was used to site experimental plots over elevated contamination areas (Figure 3.2). The final locations of experimental plots are shown in site aerial photos, Figures 2.2 to 2.7.

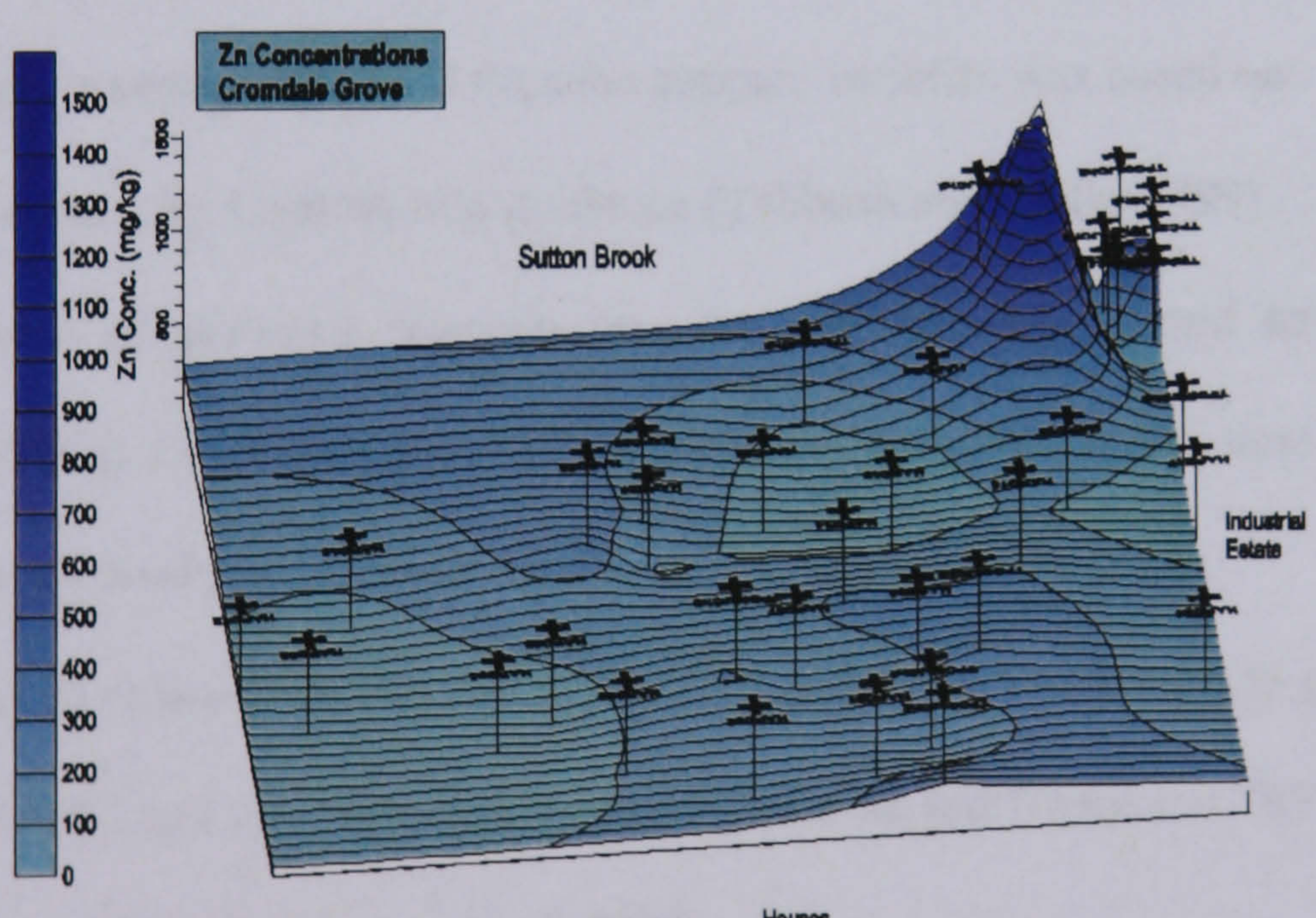


Figure 3.2. Three-dimensional representation of zinc concentrations at Cromdale Grove

3.3 Species Selection and Source

Species of interest in the present study were fast growing willow and poplar trees, on which there are several studies of their use in stabilising or remediating land contaminated with heavy metals (Landberg and Greger 1994; Riddell-Black 1994; Kumar *et al.* 1995; Punshon and Dickinson 1999; Pulford and Watson 2003; Watson *et al.* 2003). Several native species known to survive on degraded or contaminated land were also considered. *Betula pendula* is well documented to tolerate elevated concentrations of heavy metals, and is considered a hardy pioneer species (Utriainen *et al.* 1997; Kozlov *et al.* 2000). *Alnus incana* is a popular choice for urban and brownfield forestry schemes due to its nitrogen fixing properties and ability to survive on poor quality soils (Moffat and McNeill 1992; Moffat 2000). Both species were used in previous projects undertaken by JMU and UoL research groups with promising results (Mersey Forest 1999b; Putwain *et al.* 2003).

On the advice of the Forestry Authority one commercial forestry species was included. After consultation hybrid larch, *Larix* x *eurolepis,* was selected. Larch has frequently been used in restoration programs on former mine spoil sites (Bending and Moffat 1999), although its use alongside short rotation coppice taxa is the subject of some debate, as it may act as a host for certain rusts and other pathogens.

Selection of the remaining *Salix* and *Populus* coppice varieties was based on:

- Current Forestry Commission guidance (Tabbush and Parfitt 1999)
- A review of previous research into the role of willows and heavy metals, as discussed in Chapter 1. (limited research was available at the time of planting on poplar and heavy metal interactions)
- Taxa availability from local sources and suppliers and taxa used in previous studies within JMU and UoL research groups. (Punshon and Dickinson 1997; Punshon and Dickinson 1999; Rawlinson *et al.* 2000).
- Best practice guidance of selecting genetically different material within a planting area, to reduce the risk of spread of disease (Tubby and Armstrong 2002)
- The desire to plant genetically different hybrids to examine responses in the soil.

With these factors considered the species selected were as follows:

Table 3.1. Tree species selected for use in this study.

Species	Stock Type	Mix Code	Abbreviation	Peg Colour
1. *Larix* x *eurolepis*	20cm cell grown	n/a	**LAR**	n/a
2. *Betula pendula*	20cm cell grown	n/a	**BET**	n/a
3. *Alnus incana*	20cm cell grown	n/a	**ALN**	n/a
4. *Populus deltoides* x *nigra* '**Ghoy**'	25cm unrooted peg	n/a	**GHY**	
5. *Populus trichocarpa* '**Trichobel**'	25cm unrooted peg	n/a	**TRI**	
6. *Salix caprea* x *cinerea* x *viminalis* '**Calodendron**'	25cm unrooted peg	BC	**CAL**	
7. *Salix viminalis* '**Orm**'	25cm unrooted peg	A	**ORM**	
8. *Salix caprea* x *viminalis* '**Coles**'	25cm unrooted peg	AZ	**CLS**	
9. *Salix burjatica* '**Germany**'	25cm unrooted peg	XY	**GER**	
10. *Salix viminalis* x *schwerinni* '**Tora**'	25cm unrooted peg	A	**TOR**	

Species 1-3 were acquired from Cheviot Trees Ltd. as 20-30cm cell grown stock

Species 4-6 were obtained from A F Hill and son as 25cm unrooted pegs.

Species 7-10 were obtained from the Ness Botanic Garden Willow Collection as 25cm unrooted pegs.

Throughout this study species or taxa may be referred to by the abbreviations shown in the table above.

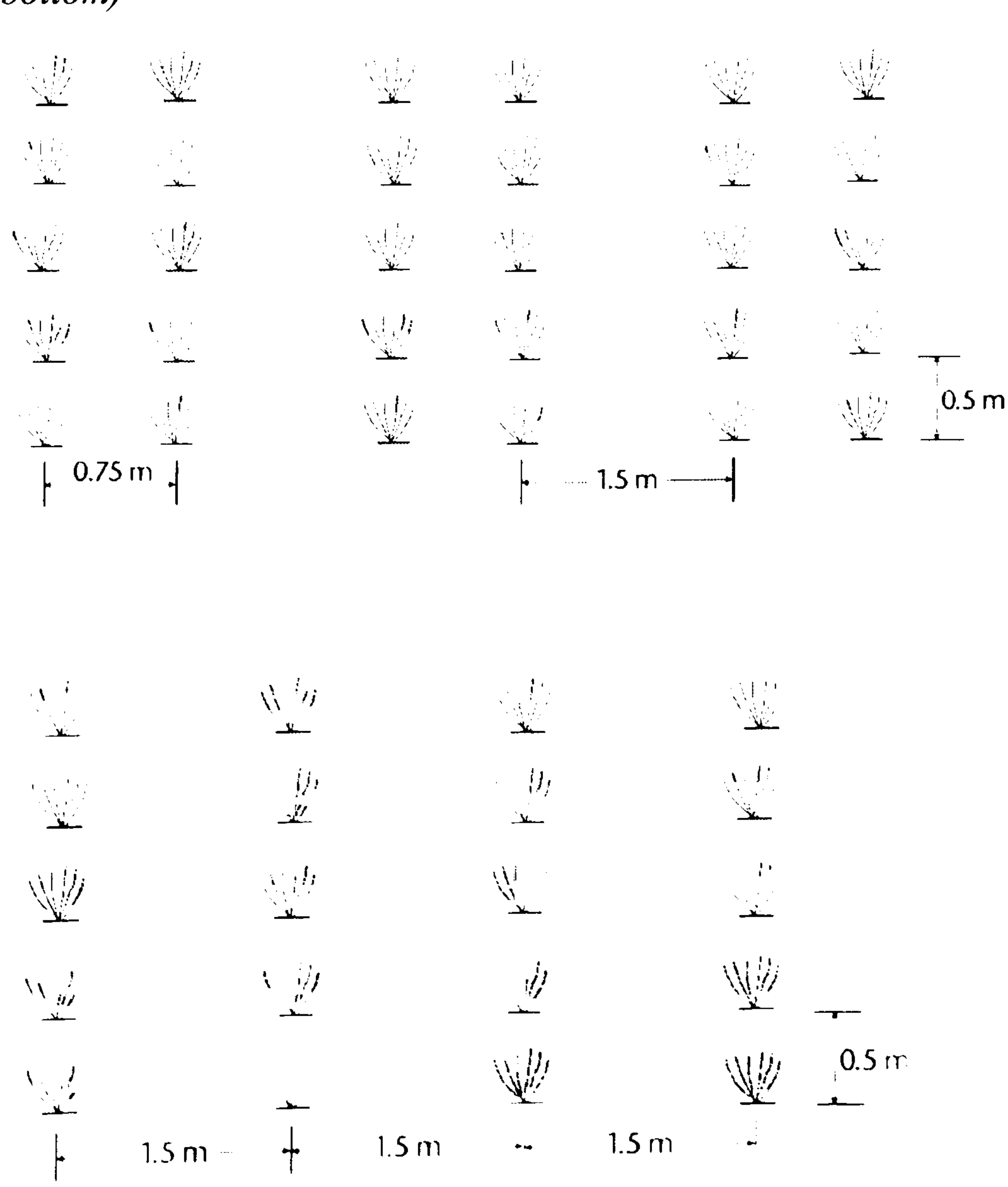
Willow and poplar pegs were colour coded on the top of the peg using forestry spray paint for identification. They were graded by diameter, collated into bundles of 50, and stored in a cold room (4°C) in polythene bags until needed. Bags were rotated periodically to prevent moisture build up.

3.4 Plot Design and preparation

Plots were smaller then those used on the previous Landfill Project (Rawlinson *et al.* 2000) and more suitable for the closer spacing of trees associated with short-rotation coppice. A standard plot size of 30m x 30m was used, although was reduced on certain sites to 21m x 21m to meet planning, statutory or local design requirements

Consistent with best practice guidance at the time of planting, willow and poplar coppice should be 1.5m between rows and 0.5m within rows (Armstrong *et al.* 1999). This spacing was adopted for all experimental taxa, including non-coppice species, although planting is in single rows, not double as is typical of many coppice plots (Figure 3.4). Buffer zones surrounding experimental planting were used at Cromdale Grove and Sugar Brook.

Figure 3.4. Recommended spacing of SRC (top) and adaptation used in this experiment (bottom)



3.4.1 Ground Preparation

Site preparation for SRC species is different to that for traditional cell grown and nursery stock. Success and establishment of the pegs is dependent on good quality ground preparation. Guidance for site preparation was published in 1999 in Forestry Commission Practice Note 7: Establishment of Short Rotation Coppice (Armstrong 1999), although some aspects of this guidance were updated in 2002 (Tubby and Armstrong 2002). This protocol would require adaptation to meet the specific needs of the project, whilst still ensuring the success of the trees. It was therefore necessary to combine this guidance with other best practice information and research on preparation of derelict or brownfield sites. All protocols for site preparation of SRC are based on agricultural land, but previous experience has implied other factors, such as compaction to be a major impediment to tree growth and recommends total cultivation where possible, especially if amendments are being incorporated (Bending and Moffat 1999; Rawlinson *et al.* 2000). Other general site impediments for tree growth are discussed in Chapter 1 and in Moffat and McNeil (1994).

3.4.2 Spraying

Herbicide spraying was not possible before the winter as specified due to timescales of site and plot selection. All plots were sprayed with glyphosate before cultivation took place, although poor weather conditions meant spraying was late and had not fully taken effect when cultivation and planting commenced.

3.4.3 Cultivation

Total cultivation is recommended for SRC preparation and is considered good practice for brownfield sites undergoing tree planting schemes. However, deep cultivation may have buried any contamination of interest as many of the sites are likely to have contamination within the top 500mm of soil. Ripping was used on sites with perceived drainage problems.

and also where the wider planting scheme required furrows in which to plant tree material. Cultivation was carried out with a JCB 3X turning the soil to a depth of approximately 300mm.

3.4.4 Power Harrowing / Rotovating

A final finish with a power harrow was recommended, but field observations suggested a rotovator would be of more use, especially in breaking up large clods of earth left after excavation due to the slow and late action of the herbicide. Rotovating was carried out after the excavation, or on some plots was used beforehand to break up clods of grass / earth. Whichever method was employed the resultant fine tilth finish was the same.

3.4.5 Fencing

All plots were fenced following or during cultivation to a standard deemed necessary on the site. Fazakerly, Sugar Brook and Kirby Moss were enclosed using rabbit wire. Merton Bank was stock (3 wire) fenced, principally to prevent access by the general public. Cromdale Grove had a 3 wire fence installed, although this was later upgraded to rabbit fencing.

Plate 3.4. Site preparation on Merton Bank 1 (top) Cromdale Grove 1 (bottom).
The JCB turns the soil to 300mm whilst the tractor mounted rotovator creates a fine tilth finish.

A summary of the cultivation regimes used on each plot is shown in Table 3.2

Table.3.2. Summary of cultivation regimes at the Brownfield sites.

Site	No of plots	Total Plot Planting Area (ha)	Tractor rip @ 2m	Crawler rip @ 2m	crawler rip @2m & total cult. 300mm	Total cult. To 300mm (no rip)	Rotivate	Flail	Strim	Fencing	Total pre-plant spray (glyphBiactive)
Sugar Brook	2	0.18	×	×	✓	×	✓	✓	×	Rabbit	✓
Fazakerley	2	0.1341	×	×	×	✓	✓	×	×	Rabbit	✓
Merton Bank	3	0.2241	×	×	×	✓	✓	×	✓	Stock	✓
Cromdale Grove	2	0.18	×	×	✓	×	✓	✓	×	Post / 3 wire	✓
Kirkby Moss	1	0.044	×	×	×	✓	×	×	×	Rabbit	✓
Corus	1	0.045	×	×	×	×	✓	×	×	Rabbit	×

3.5 Site Amendments

Amendments were applied to some plots to investigate if they provided a measurable change in soil-plant metal mobility. Where amendments have been applied this has been with the approval of the project team, landowners and other statutory authorities. In most cases soils were sampled pre- and post-amendment.

Fazakerley – Plot 1

This plot is located alongside a known copper-contaminated watercourse that flows through the site. This offered the possibility of future irrigation of the plot with water taken from the channel, prior to it reaching a reed bed treatment system established to improve water quality and remove metals from the stream. The proposed irrigation programme was not started during the project and no amendment was applied.

Fazakerley – Plot 2

With the co-operation of North West Water (United Utilities) this plot was treated with two types of processed organic waste; sewage cake and sewage sludge. Control areas were maintained with no amendments (*see* Experimental Design). Approximately 15 tonnes of each organic waste was applied in two replicate treatment areas within the plot, and then incorporated into the top 300 mm of soil. The use of processed sewage materials creates an opportunity to investigate an increased metal loading and the effect of increased productivity from organic amendments (Bardos *et al.* 1999; Moffat *et al.* 2001.; Pulford *et al.* 2002).

3.6 Experimental Design

Construction of the experimental planting design within the plots was based on the following criteria:

- **Reproducibility** – A comparable design was required across all plots
- **Replication of planting material** – The selected taxa were sufficiently replicated to allow some mortality without unduly affecting or compromising the value of the data to be collected.
- **Randomisation** – The allocation of species within each block was fully randomized.
- **Current guidance** – The design followed, as far as possible, current guidance and best practice for the cultivation and management of the selected taxa.
- **Sampling options** – Plots were designed to allow a series of sampling options in the future.

Diagrams of a range of plot designs can be found in Chapter 4, and should be referred to throughout the following sections. Full details of all plot experimental designs can be found in Appendix 1.

Basic experimental design was based on a 30m x 30m plot. This allowed the creation of 100 blocks within each plot. Each block contained 12 plants of the same taxa, in two rows of 6 trees (1.5 m between rows, 0.5 m within rows). This provided 10 replicate blocks of each taxa within each plot. Planting in double rows was intended to mimic recommended planting practices and allowed soil sampling and measurement of environmental variables between rows of the same taxa. Blocks of were randomly allocated within the plot.

This design template was established on the following plots:

- Sugar Brook 1

- Cromdale Grove 1
- Cromdale Grove 2
- Merton Bank 1
- Merton Bank 3

The basic design was modified, as described below to allow the remaining plots to be reduced in size to comply with site constraints, treatments and sampling requirements.

Fazakerley 1

Plot size 21m x 21m. Four treatment blocks were created each containing 10 species blocks consisting of 12 trees. However, the 12 trees within each treatment block were spaced in 3 rows of 4 trees. The 4 treatment blocks allowed the possibility of two to be irrigated and two as controls.

Fazakerley 2

Plot size 30m x 30m. The plot was divided into 7 treatment blocks, each containing 10 species blocks (1 block of each species, randomised with the treatment block). Each species block consisted of 18 trees, spaced in 3 rows of 6 trees. Treatments in each block consisted of 2 sewage sludge, 2 sewage cake and 3 control blocks with no amendment, but still cultivated to 300mm.

Sugar Brook 2

Plot size 30m x 30m. The plot was essentially the same design as the standard 30 x 30 plot, with 100 species blocks, and each species block replicated 10 times across the plot. However, the plot was divided into 4 treatment blocks, each a quarter of the plot and containing 25 species blocks. Two of the treatment blocks located diagonally to each other received river dredgings. All blocks were cultivated to 300mm. Each treatment block contained 2 duplicates of each species, totaling 20 blocks. Species were randomised within each treatment block.

Kirby Moss 1 and Merton Bank 2

Plot sizes 21m x 21m. These plot sizes were reduced to comply with site access and planning restrictions. Each plot had a total of 70 species blocks, each species was replicated 7 times. Each species block consisted of 9 trees, spaced over 3 rows of 3 trees.

A summary of plot sizes, amendments and tree numbers is provided in Table 3.3

Table 3.3. Summary of plot design information

Site	Plot No.	Size (m x m)	Planting Date	Blocks / Species replication	Times each species block replicated	Amendment	Total Trees
Sugar Brook	1	30 x 30	03/04/2000	100 blocks - 12 per block, 2 rows of 6	10	n/a	1200
	2	30 x 30		100 blocks - 12 per block, 2 rows of 6	10	River Dredging - 2 areas	1200
Faz Reed Beds	1	21 x 21	03/04/2000	40 Blocks - 12 per block	4	n/a	480
	2	30 x 30		70 Blocks - 18 per block	7	Sewage Sludge and Cake	1260
Cromdale Grove	1	30 x 30	04/04/2000	100 blocks - 12 per block, 2 rows of 6	10	n/a	1200
	2	30 x 30		100 blocks - 12 per block, 2 rows of 6	10	n/a	1200
Merton Bank	1	30 x 30	04/04/2000	100 blocks - 12 per block, 2 rows of 6	10	n/a	1200
	2	21 x 21		70 blocks - 9 per block	7	n/a	630
	3	30 x 30		100 blocks - 12 per block, 2 rows of 6	10	n/a	1200
Kirby Moss	1	21 x 21	20/04/2000	70 blocks - 9 per block	7	n/a	630
						Total	**10,200**

3.6.1 Planting

Planting of all plots took place between the 3rd – 20th April 2000 and was conducted by Lowther Forestry under the supervision of the research team and the Mersey Forest. Willow and poplar pegs were planted by hand, pushed directly into the ground so that approximately 1/8 of the peg remains above the surface (Dickinson *et al.* 2000) Cell grown stock were planted using notch planting techniques (Forestry Commission 1991).

3.6.2 Pot trials

To compliment field experiments a series of pot trials were commissioned. Soils were collected from Fazakerly and Merton Bank sites, and after homogenisation using a cement mixer were been placed into twenty 15 litre containers for each site. In addition to site soils, 20 pots of sterilised loam from University of Liverpool Ness Botanic Gardens were established as controls. Each container was planted with one of each experimental species, allowing each taxa to be replicated. These trees were irrigated throughout the year using a drip irrigation system. Forced re-location of this experiment caused a strong bias in later growth seasons thus the trial was partially abandoned and results are not presented within the present study.

Plate 4.2. Pot trials situated on the roof enclosure at JMU

3.6.3 Post planting follow-up

Sites were checked one week post planting to correct minor planting errors or incorrectly placed pegs. Trees uprooted by vandalism on Merton Bank and Cromdale Grove were replaced, although replacement numbers were not significant. No further tree replacement occurred after this time.

3.6.4 Changes in Plot Status

Cromdale Grove, Plot 2 (located on the Western side of the site, adjacent to Parr Industrial Estate) was severely damaged by rabbits or hares in Summer 2000. Approximately 70% of stock on the plot was severely damaged or destroyed. The site was later enclosed by rabbit fencing and some stock regenerated. However, due to the damage this plot was excluded from the study. Plot 1 on Sugar Brook was also damaged by rabbit incursions through damage to the fence. Tree losses were not significant, although it may have led to a reduction in Year 1 yield.

3.6.5 Weed Control

Weed control was carried out in June 2000 following a wet spell which had promoted weed growth on all sites. Following advice from the Forestry Commission (Willoughby and Clay 1999) and David Clay Associates, the following herbicide mixes and application methods were implemented on all plots:

Table 3.4. Weed control measures used in June 2000

Application Method	Herbicide	Supplier	Target species
Directed spray between rows	Challenge (glufosinate-ammonium)	AgrEvo	All
Entire plot	+ Dow Shield (clopyralid)	Dow Elanco	Dicots
Entire plot	+ Falcon (propaquizafop)	Cyanamid	Grasses

+ Used together in tank mix.

Strimming was also employed on sites with particularly virulent weed growth such as Sugar Brook. The effectiveness of the Dow Shield / Falcon tank mix was not as good as expected and weed control in 2001 and 2002 seasons involved a combination of strimming and directed application of glyphosate.

3.7 Field Sampling Methods

3.7.1 Soil Sampling

The primary reason for soil sampling was to accurately assess and quantify the background concentration and dispersion patterns of potentially toxic heavy metal elements in soils on experimental plots. Each soil sample was analysed for Arsenic (As), Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb) and Zinc (Zn). In addition Chromium (Cr), Mercury (Hg) and Selenium (Se) analysis was planned but not conducted due to the analytical equipment available.

3.7.2 Sampling Plan

Subsequent to planting and initial establishment surveys a design was produced to sample the soils from each plot, based on the following guidance and research documents:

- DoE CLR 4 Sampling Strategies for Contaminated Land (DoE 1994)
- British Standard BS10175:2001, Investigation of potentially contaminated sites, Code of practice (BSI 2001)
- British Standard BS ISO 10381-1:2002, Soil Quality Sampling - Part 1, Guidance on the design of sampling programmes (BSI 2002).

Sampling points were distributed in a herringbone design, allowing an optimum number and spacing of samples to be taken in order to achieve the most comprehensive overview of contaminants within each plot. The formula takes into account likely shape of contamination hotspots, size of plot, size of hotspot the user wishes to detect, and possible number and location of hotspots (DoE 1994).

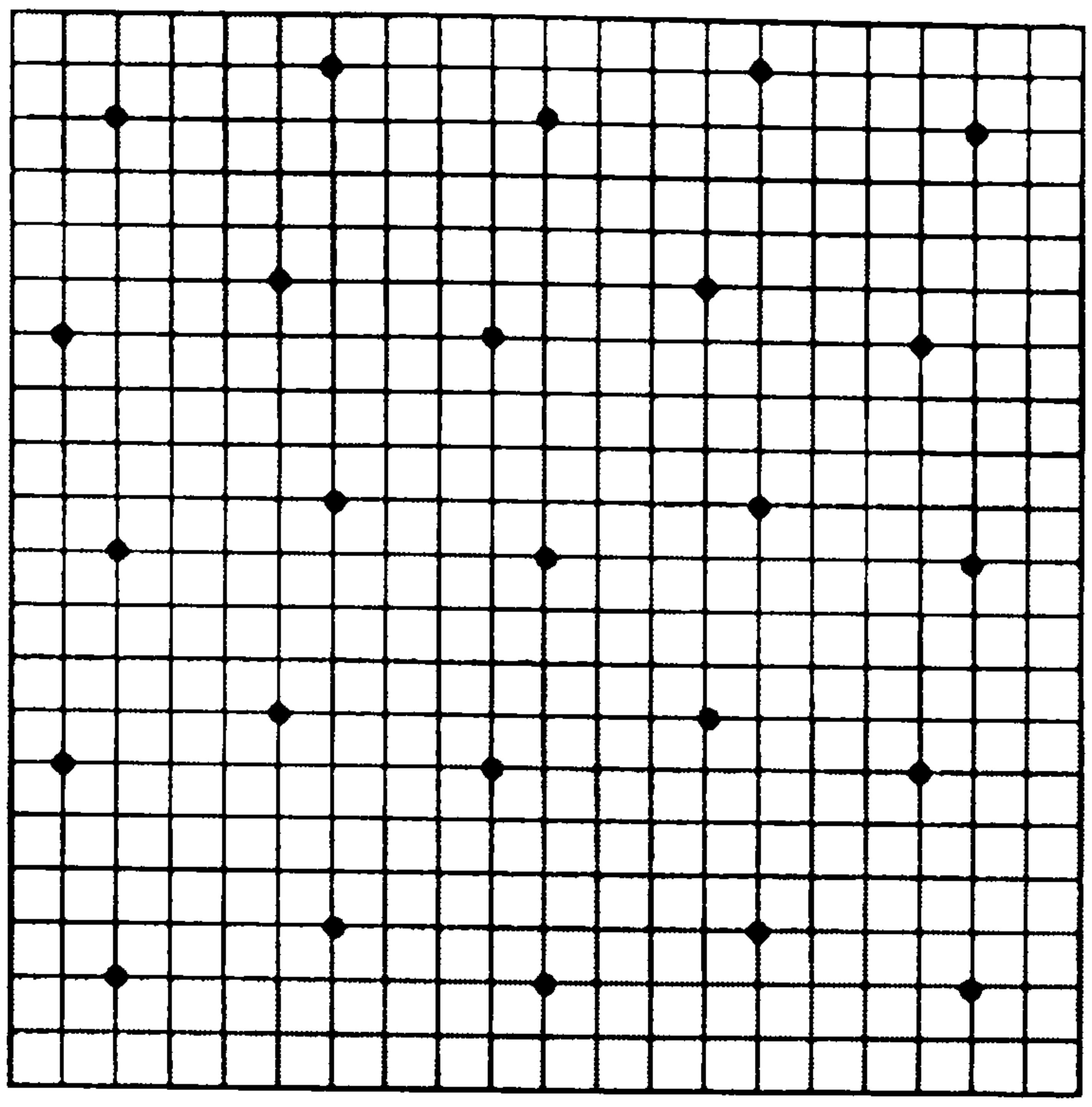


Figure 3.5. Herringbone soil sampling design used on experimental plots

Using this method it was calculated that 72 soil samples from full-size 30 x 30m plots would be sufficient to detect a hotspot of 1% of the plot size (3m x 3m), assuming hotspot size and location are unknown. The same formula was applied to smaller plots, allowing the number of sampling points to be reduced whilst maintaining a robust protocol and hotspot detection size. Soil sampling plans are shown in Chapter 4.

Soil samples for initial baseline contamination analysis were collected prior to planting in January to March 2000; referred to as Year 1 soil samples. Samples were collected at 0-30 cm depth, although in some areas there was insufficient soil to collect to this depth. Where this was the case the depth of soil removed was recorded. Samples were taken using a 2.5 cm soil auger, bagged and returned to the laboratory where they were air dried at room temperature, ground using a mortar and pestle, and sieved to a <2 mm fraction. The need for good field technique to collect samples from each plot that could be compared and reproduced led to the production of a protocol for soil sampling throughout the project. Each sample was catalogued and stored in cool, dark conditions. These samples were also used for analysis of physical and chemical parameters.

3.7.3 Soil Analysis

All Year 1 soil samples were digested in Analar grade concentrated HNO_3 and analysed using Atomic Absorption Spectrophotometery (AAS) as described in Section 3.9. A subset of these samples were also analysed for EDTA and $CaCl_2$ extractable metals as described in Section 3.10. Samples to be analysed by this method were selected on the basis of locating hotspot areas of soil contamination as described in Chapter 4.

3.7.4 Contaminant Mapping

Results from Year 1 soil metal analyses were used to produce contour maps of contamination distribution and concentration at each plot, using the SURFER package (Golden Software). Full results of this exercise are shown in Chapter 4. Maps indicate total metal concentrations and not plant-available; their main purpose was to identify target areas where high concentrations occurred and to focus on these locations where future experimental work would be directed. The maps made it possible to identify trees growing on areas of highest contamination, and to target these trees for future intensive investigation. Hot spots identified in this analysis were also analysed for measures of 'bio-available' metals (using EDTA and $CaCl_2$ extraction).

3.7.5 Bulked Soil Samples

A bulked sample, representative of the entire plot or treatment was obtained by combining sub-samples from individual Year 1 soil samples. The number and size of samples used to create a bulked sample varied between plots and treatments. In plots where there were multiple treatments or amendments, bulked samples were made for each experimental treatment. Bulked samples were not used for metal analysis but for physicochemical parameters including Particle Size, pH, Organic Matter and Total Nitrogen.

3.7.6 Year 3 Soil Sampling

Additional soil sampling was conducted in January and February 2003 immediately after final tree harvesting. Soils were re-sampled from points identified as hotspots using the same protocols described previously.

3.7.7 Foliar Sampling

Foliar sampling was carried out initially in September 2000. Leaf samples were taken from all but the outer two trees in each species block, which provided a buffer zone. For example, in 30m x 30m plots, where each species were in blocks of two rows of 6 trees, only the inner 4 trees in each row were sampled (8 trees in total). Foliar sampling was conducted based on protocols in (UN/ECE-EC. 1998). Only fully open leaves were collected from the top third of the tree, with equal numbers of leaves collected from each compass ordination point. Foliar samples were oven-dried (80°C) and stored for potential future analysis, depending on the results of the contamination mapping exercises; analyses were later directed towards trees growing near hotspots. Foliar sampling was conducted on an annual basis *(see Section 5.1).*

3.7.8 Tree Census

A census of tree mortality, establishment and growth was conducted during the winter of each growing season, specifically; January 2001, February / March 2002 and November / December 2002 (for 2003 growth data). Trees on the edges of species blocks were not included due to their edge and buffering effects; as above, only the inner 8 trees of each block were assessed in a standard 30m x 30m plot.

Assessment was made by measuring the total height of the tree, from ground level to the tip of the tallest stem. Trees that were dead, or simply not present were recorded as such.

3.7.9 Cutting Back

Immediately after height data was recorded for Season 1 all coppice species (*Salix* and *Populus*) were cut back in line with current guidance for maintenance and establishment of Short Rotation Coppice (Armstrong 1999). This process constituted cutting all main stems of the tree approximately 2-3 cm above ground level and was conducted by hand cutting using lopping shears (Plate 4.3). Cutting back reduces apical dominance allowing re-growth during the next growing season to produce multi-stemed trees and potentially to increase productive biomass.

After this process all willow and poplar were allowed to grow for 2 full growing seasons before any further destructive sampling of stems.

Plate 4.3. Cutting back coppice stools of willow and poplar at the end of year 1

3.7.10 Stem sampling

Before harvesting of all stems at the end of Year 3 for biomass determination, trees from hotspot areas to be analysed for metal content were tagged. This enabled stems to be used as part of the biomass determination process to give accurate field results, but subsequently allowed easy removal and transport back to the lab for metal content analysis.

3.7.11 Biomass measurement

Determination of tree biomass growth was conducted at the end of Year 3 for each species with the exception of larch, to give an accurate measure of growth over 2 full growing seasons. Each species block was felled in turn using a chainsaw, removing all stems at 2-3cm above ground level. The contents of each block were transferred to an adjacent tared weighing bin mounted on a set of weighing beams (Plate 3.5), calibrated to 0.1kg accuracy. Total weight for each block was recorded before a composite sub-sample from each species was collected for immediate moisture determination. In the laboratory, sub samples were pooled for each species and weighed to 2 decimal places on a flat pan balance before being dried at 105°C to a constant weight, calculating moisture content as a percentage of total fresh weight. This correction factor was applied to the field recorded weights and averaged for all species within the plot before being converted to give a standard measurement of oven dry tones per hectare (ODT ha^{-1}).

Plate 3.4. (left) Harvesting operations for biomass determination

Plate 3.5. (right) Field weighing equipment used for biomass determination.

3.8 Laboratory Preparation of Samples

Soils

Soils were air dried in the laboratory at room temperature until no further change in weight was detected and ground using a decontaminated mortar and pestle before being passed though a 2mm stainless steel sieve. Soils were stored in airtight ziplock bags in the dark until required. All soil analysis was conducted on air dried material, unless otherwise stated.

Foliar

Foliar samples were oven dried at 80°C prior to being ground. The same bag was used from sample collection, drying and grinding to prevent cross contamination.

Stem

Three stems were selected at random from each harvested tree. Ten cm sections were cut from each stem at 50cm intervals along its length and washed in distilled then deionised water and dried at 80°C. Samples that required bark and wood material to be analysed separately involved an additional step of bark removal that was conducted after drying using a stainless steel scalpel blade to remove bark from the stem. All material was ground and homogenised using a laboratory mill.

3.9 Environmental Analysis laboratory methods

All acids and reagents used were analytical quality. All glass and plastic ware was washed in 2% decon before washing in 3% HNO_3 acid wash and rinsed in deionised water. Chemical analysis was carried out in duplicate unless otherwise stated.

3.9.1 Microwave digestion of environmental samples

All acid digestion of environmental samples was conducted using a MARS 5 microwave digestion unit from CEM Corporation. This method allows rapid digestion of products in a reproducible and controlled environment, temperature and pressure measurements are monitored and maintained for each run, improving quality control. The use of a closed system minimises sample loss or cross contamination and ensures constant heating for all samples. Specially designed PTFE vessels are used for sample digestion, which are pressure sealed and mounted in support structures prior to loading into the digester unit. Reagent blanks were run with each batch of 12 samples.

Microwave Soil digestion

All Year 1 soil samples were analysed for pesudo-total extractable metals by microwave digestion in HNO_3 and HCl, using a method closely adapted from US Environmental Protection Agency (USEPA) Method 3051 and 3051a 'Microwave assisted digestion of sediments, sludges, soils and oils' (USEPA 1994). Air dried soil (0.5g) was weighed to 4 decimal places into microwave PTFE vessels and 9ml concentrated. HNO_3 plus 3ml concentrated HCl were added to each sample. Samples were heated to 195°C in 10 minutes and held at this temperature for a further 10 minutes. Once cool, samples were filtered though a Whatman 540 filter paper and made up to 25ml with deionised water prior to metal determination.

Microwave Foliar, stem and bark digestion

0.5g of dried and homogonised foliar, stem or bark material was weighed into microwave PTFE vessels and 8ml concentrated HNO_3 plus 2ml H_2O_2 added to each sample. Samples were heated to 185°C over 10 mins and held at this temperature for a further 20 mins. Once cool, samples were filtered though a Whatman 540 filter paper and made to 25ml with deionised water prior to analysis.

3.9.2 Soil pH (UNEP / UNECE 1997b)

Air dried soil (10g) was weighed into a polypropylene bottle and 50ml distilled water added before shaking for 1 hour on a end-over-end shaker. A calibrated HANNA pH meter, fitted with a glass bulb electrode was used to record pH (H_2O). Each sample then received 4ml 0.125M $CaCl_2$ solution (in order to make a 0.01M $CaCl_2$ solution). The shaking and analysis process was repeated and recorded as pH ($CaCl_2$).

3.9.3 Total Nitrogen (Kjeldahl method) (Allen 1989)

Composite soil samples from each plot were analysed for total nitrogen using the Kjeldahl method (Allen 1989; Gerhardt Ltd 2002) using a Gerhardt digester block and Vapodest 20 distillation unit.

1g of air dried soil was weighed into a Pyrex digestion tube and 10ml of 98% H_2SO_4 (N free) plus 1 Kjeletab CX added to each sample before heating in a digestion block at 100% power for 5 mins, 0% power for 5 mins and 70% power for 40 mins. Samples received 70ml distilled water and 60ml NaOH before distillation under forced steam pressure into 50ml boric acid solution with indicator. The boric acid solution was back titrated with concentrated HCl and total nitrogen calculated.

3.9.4 Organic Matter

5g of bulked soil sample from each plot were weighed into a porcelain crucible and placed in a muffle furnace for 6 hours at a temperature of 450°C, before removal and cooling in a desiccator. Crucibles were re-weighed and organic matter calculated as a percentage of weight loss.

3.9.5 Particle Size analysis (UNEP / UNECE 1997a)

Soils with a pH of >6.8 required a pre-treatment step to remove carbonate. 20g soil and 100ml acetate buffer were heated on a water bath to 100°C and buffer added in 25ml increments until effervescence stopped. After cooling and settling the supernatant was removed. Organic matter was removed by the addition of 15ml increments of 30% H_2O_2 on an 80°C water bath. Once complete 300ml of water was added and boiled gently to remove any remaining H_2O_2. Contents were allowed to settle and the supernatant removed.

Soil was transferred into a 1l polythene bottle and 20ml dispersing agent (4% $(NaPO)_6$ plus 1% Na_2CO_3) and 400ml water was added before shaking on an end-over-end shaker for 16 hours. The solution was passed through a 63μm sieve into a 1l measuring cylinder and made to 1l volume. Soil remaining on the sieve was dried at 105 °C before fractional sieving for sand contents. Silt and Clay fractions were measured by pippeting 20ml of solution from sedimentation cylinders at temperature dependant predefined intervals and depths as indicated in the reference method (UNEP / UNECE 1997a). These fractions were dried at 105 °C and % sand (<63 μm), silt (63 – 2 μm) and clay (<2 μm) calculated.

3.10 Soil extraction procedures and analysis

3.10.1 Introduction and rationale

Risk assessments of contaminated soil need to address metal bioavailability. Strong acid extractions to give total or pseudo-total metal concentrations differ considerably to the proportion of the metals available for plant uptake; these fractions are hard to predict (Boudou 1996). Reasons for this relate to the many factors affecting metal speciation in the soil system such as pH, organic matter, complexation, adsorption and other factors discussed in Chapter 1. Extraction procedures using EDTA and DTPA originally developed for the assessment of nutrient status in soils on agricultural land may not be applicable to the historical anthropogenic metal contamination found in urban soils (Dickinson *et al.* 2000). A number of different extraction procedures have been suggested and are currently in use for assessing potentially bioavailable fractions of metal contaminants in soils, despite efforts to harmonise procedures (Quevauviller *et al.* 1996b). This subject is one of considerable debate and a full discussion is beyond the scope of this study, although extensively reviewed elsewhere (Chen *et al.* 1996; Quevauviller *et al.* 1996b; Ure 1996)

The use of $CaCl_2$ as an extractant to simulate the bioavailability of heavy metals is strongly advocated as it matches soil solution with respect to pH, concentration and composition (Houba *et al.* 1996 ; Novozamsky *et al.* 1993) and is set to become a standard technique in some parts of Europe (Pueyo *et al.* 2003). Since one of the main factors for actual plant availability of metals in soils is pH, the use of neutral buffer solution is warranted as opposed to strong acid digests. $CaCl_2$ has been correlated with uptake in above ground plants, but the correlation was not valid at higher pH values (Brun *et al.* 1998). Different extractants will cause different impacts on different metals, and many brownfield soils may be contaminated with multiple metals. $CaCl_2$ is a very weak extractant, removing water soluble or readily exchangeable forms. EDTA will also extract organically bound metals and carbonate forms. Even when extracting the same metal, different site, plant and soil interactions can generate differing results. Brun found $CaCl_2$ to be the best indication of Cu uptake into plants, where as Song *et al.* (2004) found 1M NH_4NO_3 or DGT more indicative of plant uptake compared to $CaCl_2$ and EDTA.

EDTA is a common extractant and has been used extensively in estimating plant available concentrations as a single solution, but also as part of sequential extraction procedures. A short laboratory trial conducted as part of this study showed it was easier to use than DTPA, as recoverable quantities generated for analysis were higher. Although it may have limitations with respect actual plant available metals, this is compensated by its ease of use, wide variety of comparable data in other studies and the opportunity to provide new data on its behavior and practical use on a range of urban brownfield soils with multiple metal contaminants.

Sequential extractions are common, but can be time consuming. Therefore a modified version of a short extraction procedure was adopted for this study. Soils were extracted with $CaCl_2$ according to Maiz *et al.* (1997), but the second phase, originally using DTPA was replaced by an extraction procedure using EDTA as described in Quevauviller *et al.* (1996a), providing mobile ($CaCl_2$) and mobiliseable (EDTA) fractions. Full method details are provided below.

3.10.2 Soil sequential Extraction Procedure

Three grames of air dried soil was weighed into a 50ml centrifuge tube and 30ml of 0.01M $CaCl_2$ added. Samples were shaken using an ELE laboratory end over end shaker for 2 hours. Samples were then centrifuged for 15 mins at 3000g before 25ml of supernatant was filtered though pre wetted Whatman 42 filter papers into sterile universals. Any remaining supernatant was discarded taking care not to discard any soil. To each sample 30ml 0.05M EDTA solution was added and the samples were redistributed by shaking for a further 1 hour before centrifuging, pipeting and filtering as described above. Samples were stored at 4°C prior to analysis

Soils samples from year 3 were not analysed for $CaCl_2$-extractable metals due to time constraints and very low recoveries, approaching detection limits, for many metals. This is discussed in Chapter 5.

3.10.3 Metal Analytical equipment

Analysis of all vegetation material and soil metals extracted using the $CaCl_2$ / EDTA process was conducted using Inductively Coupled Plasma – Optical Emission Spectrophotometery (ICP-OES). Sample analysis of Year 1 total soil metals extracted by HNO_3 / HCl were analysed by Atomic Absorption Spectrometry (AAS).

Year 1 $CaCl_2$, EDTA soils and Year 2 foliar samples were analysed using a Spectrohm ICP-OES based at the Environmental Analysis Laboratory at Forest Research, Farnham, Surrey. Year 3 soil EDTA, foliar and stem samples were analysed on a Perkin Elmer Optima 3300 RL based at the NERC Analysis Centre, Royal Holloway, University of London. AAS determination was conducted using a Perkin Elmer AAnalyst 100 at Liverpool John Moores University, Biological and Earth Science department.

For all analytical work, matrix matched standards were prepared and used for standardization or matrix correction. Reagent blanks were analysed approximately every 12 samples, and standard solutions analysed every 10 samples to check for instrument drift. In addition certified reference materials (CRMs) for known extraction procedures were used for HNO_3 soil analysis and all vegetation analysis at a frequency of approximately one every 25 samples. Yttrium, at a concentration of 20ppm was added to samples analysed at the Forest Research as a further internal quality control check.

3.11 QA / QC Analytical testing

Because ICP analysis of foliar, stem, EDTA and $CaCl_2$ soil samples occurred at 2 different locations on 3 different time periods, it was critical to use Certified Reference Materials (CRMs) to ensure results were reproducible and comparable.

CRM, NCS DC73349 'bush, branches and leaves' was obtained from Laboratory of the Government Chemist (LGC). CRM's were prepared in the same manner as other samples, approximately every 25 – 30 samples.

Table 3.5 shows a summary of results from CRM analysis during analytical runs for foliar and stem samples. Table headings indicate separate runs conducted at Forestry Research (FR) and the NERC ICP facility (NERCx, where x refers to independent sampling times). Numbers of decimal places used are derived from CRM concentrations as listed in the certificate of analysis. CRM's in square brackets are non certified values only indicative values as shown on the CRM certificate of analysis.

Table 3.5. Results of QC checks on plant material samples using Certified Reference Material NCS DC73349 on 3 different occasions.

	FR	NERC 1	NERC 2	FR	NERC 1	NERC 2	FR	NERC 1	NERC 2
		As			**Cd**			**Cu**	
CRM		**1.25 (0.1)**			**n/a [0.38]**			**6.6 (0.4)**	
Mean	**1.57**	**1.25**	**1.05**	**0.78**	**0.79**	**0.88**	**6.6**	**6.9**	**6.9**
Min	*0.72*	*0.14*	*0.65*	*0.72*	*0.73*	*0.85*	*6.2*	*5.5*	*5.2*
Max	*2.89*	*2.34*	*1.80*	*0.90*	*0.84*	*0.91*	*7.7*	*8.0*	*8.4*
n	*13*	*11*	*7*	*13*	*11*	*7*	*13*	*11*	*7*

	FC	NERC 1	NERC 2	FC	NERC 1	NERC 2	FC	NERC 1	NERC 2
		Ni			**Pb**			**Zn**	
CRM		**1.9 (0.2)**			**47 (2)**			**55 (2)**	
Mean	**2.0**	**1.8**	**1.8**	**51**	**50**	**50**	**53**	**54**	**55**
Min	*1.3*	*1.3*	*1.5*	*47*	*44*	*42*	*50*	*45*	*43*
Max	*3.2*	*2.1*	*2.5*	*54*	*55*	*55*	*62*	*63*	*65*
n	*13*	*11*	*7*	*13*	*11*	*7*	*13*	*11*	*7*

Table headings indicate separate runs conducted at Forest Research (FR) and the NERC ICP facility (NERCx). Errors in certified values are in round brackets. Figures in square brackets are non-certified values. All figures in mg kg^{-1}

No CRM's were available for $CaCl_2$ or EDTA soil extractions and in-house attempts to create a homoginised reference material were unsuccessful. To overcome this a series of approximately 20 samples from the initial analysis at Forest Research were also tested at the NERC facility to cross calibrate, showing no significant deviations.

3.12 Statistical and computational analysis

SPSS v11 was used for statistical testing of ANOVA, t-test and correlations. CANOCO v4.5 for windows was used for Principal Component Analysis and Microsoft Excel was used for producing ordination bi-plots and elliptical standard deviations from CANOCO output. SURFER v7 from Golden Software was used to create contour maps of contamination.

Chapter 4: Contaminant Mapping and Site Characterisation

4.1 Introduction

The aims of the site investigation were to accurately measure, describe and evaluate soil contamination and physico-chemical parameters and provide spatial profiles for each plot as a baseline for further monitoring and experimental work. Further consideration was given to the relevance of this data in the context of current UK guidance relating to land contamination.

Detailed information on soil contaminants was required:

1. To establish presence or absence of specific contamination types on the plot;
2. To facilitate collection of reliable baseline data and as the basis for further investigations for this and future projects;
3. To identify elevated hotspot areas and allow a targeted approach of selecting smaller experimental areas of each plot for more detailed investigation of soil / plant interactions.

4.2 Sampling protocols - theory

Within a commercial framework the quantity and nature of sampling required to quantify concentrations of contaminants is frequently driven by sampling and analytical costs coupled with the level, or probability required of accurately identifying contamination (Nortcliff 2001; Ramsey *et al.* 2002). Information such as site walkovers, desk or visual inspections may influence the amount of field sampling required by making assumptions on likely patterns of contaminant distribution through a site conceptual model. In many cases it may be necessary to assume that no evidence for likely hotspots exists, especially if contaminant source areas cannot be identified, and all parts of the site should be treated has having an equal chance of containing a hot spot. This was the approach adopted in designing a sampling regime for the experimental sites in the present study, as initial sampling and desk studies, such as site histories, showed no obvious pattern of contaminant spread.

The number of samples to be taken was calculated using a formula published by the former UK Department of the Environment (now DEFRA) (DoE 1994) that allows a statistical confidence of detecting a contamination hotspot to be calculated. This formula and sampling pattern is referred to in more recently derived guidance on soil sampling (BSI 2002). The theory of detecting a hotspot of a particular size may be an inaccurate way of examining sites that have relatively diffuse contamination patterns. What is required specifically for experimental purposes is the best possible resolution, within the realms of practical achievability, to allow accurate monitoring over the experimental duration, and allow future targeted monitoring of areas of interest.

Full details of the sampling design process are described in Chapter 3. Plots were sampled and analysed for a range of total heavy metals by HNO_3 / HCl acid digestion. Results were used to create interpolated contour maps of contamination for each plot using the SURFER software package.

This mapping technique is invaluable by providing an accurate visual representation of the contaminants present at each experimental plot as well as baseline data for site characterisation. This will allow a targeted approach to future assessments of tree and contaminant interactions, by identifying genuine contaminant hotspots and trees planted within them.

Sampling at this density is unlikely to be achieved in any commercial site investigation, although it highlights the wider problem of mapping old, diffuse contaminants spread on a large scale. The issue of sampling at multiple depths is not considered in these investigations, mainly due to assumptions that contaminants are located near the surface. It is a commonly held principle that analysing for total metal content with strong acids such as HNO_3 and HCl is not be indicative of bioavailable content (Boudou 1996), or the potential for that element to pose a threat to the wider environment. This is discussed in Chapter 3 and later sampling and analysis considers these factors in some depth. However, the use of strong acid extractions and total metal concentrations is a universal method and the speed, simplicity and comparison with standard methods should be considered. In this study total metal data has been used as a screening tool to identify target areas of specific metal levels that would warrant further investigation of soil contamination by a range of different techniques.

Brownfield Contamination

Historical contamination of brownfield sites in this study may have been the result of the proximity of industrial processes since the late 18th Century, including the disposal of waste materials. Over time sites may have been redeveloped or reused for different purposes, details of which are not contained in historical records. Liverpool has only half the inhabitants it did in the 1930s, and northern England has been going though a process of de-industrialisation and migration for decades. Brownfield sites have a significantly increased chance of contamination being unevenly distributed.

At Merton Bank, a site in the present study, it is known that industrial waste was dumped around the turn of the century, although few records exist of what it was (Murphy 1979). Historical records show there have been a wide range of potentially contaminative industries in this area. Approximately 80 years later the site was landscaped and additional colliery spoil and sewage waste was incorporated. This offers the potential for a highly localised contamination hotpots of differing contamination types.

4.3 Guidelines for contaminated soils

Results from contamination investigations need to be compared to statutory definitions or guidelines to assess the level of contamination from a site and associated risks, constraints and management of the land. In the UK contaminated land was defined in 2000 under Part

IIA of the 1990 Environmental Protection Act *(see Chapter 1)*. Until recently the most frequently used set of guidelines to assess the significance of contamination was the Inter-Departmental Committee on the Redevelopment of Contaminated Land: Guidance Notes (59/83) (ICRCL 1987), which provided threshold concentrations for certain organic and inorganic substances. It defined threshold concentrations in relation to a variety of land uses, including those that were considered to be phytotoxic to plants. However, these guidelines were based partly on attempts to predict toxicological limits for clean soils under threat of being progressively contaminated, were frequently misinterpreted and were widely thought to be incorrect in relation to tree planting on contaminated sites (Dickinson *et al.* 2000)

A new package of technical guidance for contaminated land was released in March 2002. CLEA (Contaminated Land Exposure Assessment) is a risk-based system of assessing human health risks arising from long-term exposure to contaminants in soil, and was derived to integrate fully with the new statutory definitions of contaminated land. Curiously, the need for a UK risk based approach to defining trigger concentrations was proposed and outlined by Simms and Beckett (1987), although it took a further 15 years to develop a UK regulatory system. In December 2002 the ICRCL guidance document was formally withdrawn from use (DEFRA 2002b). The CLEA guidelines are now the de-facto assessment tool for defining contaminated land, and although they consider receptors such as ecosystems and controlled waters the soil metal values are derived to protect human health. Each element of concern has been assessed and a soil guideline value (SGV) produced. No SGVs have been produced for Cu, Zn and other primarily phytotoxic elements, nor has consideration been given to phototoxicity thresholds in the same way as ICRCL. This provides several problems when trying to assess contaminated sites that may be suitable for phytoremediation, bioenergy or amenity woodland.

4.3.1 Land Use and Guidance

ICRCL and CLEA specify certain land use types and derive acceptable contaminant concentrations for that use. Many of the sites used in this experiment could easily fall between these categories. ICRCL Group A (Hazardous to human health) contained land use types; domestic gardens and allotments, or parks, playing fields & open space. Group B (phytotoxic) simply denotes 'any land use where trees are used' (ICRCL 1987). CLEA considers three land uses; allotments, Residential or Industrial. Sites used in this experiment would seem to fit best into the Parks playing fields & Open Space category defined in ICRCL as many are classed as amenity or public open space. When using the CLEA guidance, the closest land use is residential, based on likely exposure scenarios. For certain elements the SGVs are sub-divided for residential with or without plant uptake. However, there are several important factors to consider when trying to define a land use and to apply SGVs.

- It could be defined that a crop (SRC) is being grown on the land, although not for human consumption. Several parts of CLEA deal with uptake by plants for human consumption. Soil to plant concentration factors for uptake of contaminants to plants are based on literature reviews of garden vegetables of interest, not trees.
- If sites are considered specifically for public recreation, for example in the context of community forestry, then dermal, inhalation and oral ingestion pathways may be just as high as for domestic garden land use. This may be especially true in urban areas where people don't have gardens and use these spaces as play areas as you would a garden with the exception of growing produce.

Research has been underway for some time by the Environment Agency to define exposure parameters for amenity and public access land, but no published guidance on this has been published to date. It must therefore be argued that whenever land is detailed for use as a public resource a 'better safe than sorry' approach may need to apply to cover all eventualities, especially as the public may be encouraged to use the site. If this is the case then several other pathways may need to be considered, such as wild fruit picking, pets using the site and motor biking. All these may increase the risk of human uptake of contaminants and it may not be feasible to build into an exposure model. The problem with ICRCL is that they are not risk based. CLEA SGVs include several risk based uptake pathways and must consider the most sensitive land use if a multi use site is realised. CLEA also allows for flexibility based on the perceived risk. For example if coppice is to be grown on land inaccessible to the public then different approach may be suitable rather than classing the land as residential, as the contaminant pathway is broken by not allowing access on the site.

4.3.2 Application of Guidelines – CLEA and ICRCL

The following tables compare the use of ICRCL guidelines to more recently derived CLEA SGVs for each contaminant of interest, indicating the differences in considerations such as land use, soil pH and the potential for plant uptake, as a consumption route.

Table 4.1.1. Comparison of ICRCL and CLEA guideline values of total Cd in soil. All values in mg kg^{-1}

Cadmium	ICRCL	CLEA
Gardens / Allotments *(a) (b)*	3	1 @ pH6, 2 @ pH 7, 8 @ pH 8
Parks and Open Space *(a)*	15	-
Residential, with uptake	-	1 @ pH6, 2 @ pH 7, 8 @ pH 8
Residential, NO uptake	-	30
Industrial	-	1400
Phytotoxic	N/a	-

(a) Threshold values (b) For CLEA this section applies to allotments only

If no vegetation is present for a consumption route then the only considered route is ingestion. However, problems may arise in amenity uses where wild edible fruits such as blackberries etc. grow, which may add a consumption pathway. At soil pH values of less that 6.5 CLEA recommends an assessment of bioavailability on a site by site basis.

Table 4.1.2. Comparison of ICRCL and CLEA guideline values of total As in soil. All values in mg kg^{-1}

Arsenic	**ICRCL**	**CLEA**
Gardens / Allotments *(a) (b)*	10	20
Parks and Open Space *(a)*	40	-
Residential, with uptake	-	20
Residential, NO uptake	-	20
Industrial	-	500
Phytotoxic	N/a	-

(a) Threshold values (b) For CLEA this section applies to allotments only

Table 4.1.3. Comparison of ICRCL and CLEA guideline values of total Cu in soil. All values in mg kg^{-1}

Copper	**ICRCL**	**CLEA**
Gardens / Allotments *(a) (b)*	N/a	N/a
Parks and Open Space *(a)*	N/a	-
Residential, with uptake	-	N/a
Residential, NO uptake	-	N/a
Industrial	-	N/a
Phytotoxic *(c)*	130	-

(a) Threshold values (b) For CLEA this section applies to allotments only (c) ICRCL notes: pH assumed to be 6.5, if it falls toxic effects will be increased. Grass may not be affected at these concentrations. These are 'worst case' scenarios in acid, sandy soils, at higher pH's phytotoxic effects are unlikely to occur at these concentrations.

Table 4.1.4. Comparison of ICRCL and CLEA guideline values of total Pb in soil. All values in mg kg^{-1}

Lead	**ICRCL**	**CLEA** [(1)]
Gardens / Allotments *(a) (b)*	500	450
Parks and Open Space *(a)*	2000	-
Residential, with uptake	-	450
Residential, NO uptake	-	450
Industrial	-	750
Phytotoxic (c)	N/a	-

(a) Threshold values (b) For CLEA this section applies to allotments only
[(1)] Values apply to the geometric mean of soil concentrations across a site

Table 4.1.5. Comparison of ICRCL and CLEA guideline values of total Ni in soil. All values in mg kg^{-1}

Nickel	**ICRCL**	**CLEA**
Gardens / Allotments *(a) (b)*	N/a	50
Parks and Open Space *(a)*	N/a	NA
Residential, with uptake	-	50
Residential, NO uptake	-	75
Industrial	-	5000
Phytotoxic *(c)*	70	NA

(a) Threshold values (b) For CLEA this section applies to allotments only (c) ICRCL notes: pH assumed to be 6.5, if it falls toxic effects will be increased. Grass may not be affected at these concentrations. These are 'worst case' scenarios in acid, sandy soils, at higher pH's phytotoxic effects are unlikely to occur at these concentrations.

At soil pH values outside 6-8 CLEA recommends an assessment of bioavailability on a site by site basis. There is some possibility of uptake related to pH, but data is not conclusive. Interestingly ICRCL did not consider Ni in its Group A hazardous to human health, only phytotoxic, CLEA defines it as harmful to human health.

Table 4.1.6. Comparison of ICRCL and CLEA guideline values of total Zn in soil. All values in mg kg^{-1}

Zinc	ICRCL	CLEA
Gardens / Allotments *(a) (b)*	N/a	N/a
Parks and Open Space *(a)*	N/a	-
Residential, with uptake	-	N/a
Residential, NO uptake	-	N/a
Industrial	-	N/a
Phytotoxic *(c)*	300	-

(a) Threshold values (b) For CLEA this section applies to allotments only (c) ICRCL notes: pH assumed to be 6.5, if it falls toxic effects will be increased. Grass may not be affected at these concentrations. These are 'worst case' scenarios in acid, sandy soils, at higher pH's phytotoxic effects are unlikely to occur at these concentrations.

4.3.3 Risk-based assessment of contaminated land

Metal concentration SGVs do not address a number of important factors associated with contaminated land, including:

- cumulative impacts (e.g. with pesticides or other toxic compounds)
- synergistic interactions between different chemicals
- uptake and redistribution of metal contaminants within ecosystems
- risks to overall food web
- risks through bio-magnification

One possibility is to use CLEA derived models and concepts in conjunction with other Risk Assessment Tools, for example those developed for commercial market, such as SNIFFER, the RBCA toolkit, or BP RISC. Human-health risk models have been expanded to qualitatively consider interactive risk issues. These include:

- Ecological-Risk Assessment
- Cumulative effects from common contaminants normally found in urban environments (e.g. blood lead)

In Canada and Australia a quantitative ecological-risk assessment (ERA) has recently been introduced. This considers, for example, rare and threatened species on the site. There is no current EC guidance for ERAs, although the Netherlands has introduced Ecological Intervention Values and considers several holistic ecosystem and ecotoxicological functional monitoring approaches (van Straalen 2002). The Environment Agency has recently proposed ERA methodology for the UK, with a discussion and consultation document (Sublethal Ecotoxicological Tests for Measuring Harm to Terrestrial Systems) released in December 2003 (Environment Agency 2003). Their stated aim is to develop a coherent and consistent approach for assessing risks to ecosystems from contaminated soil.

Perhaps the best interpretation of the current state of the art is to suggest that a combination of risk assessment models and methodologies supported by the latest relevant research and guidance provide a comprehensive risk assessment for contaminated sites. In an increasingly litigious framework of 'toxic tort' the emphasis will be to prove that no significant risk is present from site contaminants if the public are to be allowed unrestricted access.

4.4 Contamination data analysis and presentation

Results from the analysis of soil samples collected from each plot are presented in Tables 4.2 to 4.9. These data were used in order to create interpolated contour contamination maps of each plot for the range of elevated metals: As, Cd, Cu, Ni, Pb, Zn. In several plots, where elements were below or at limits of detection, especially concentrations of As and Cd, maps were not constructed. All soils were mapped for Cu, Ni, Pb and Zn.

Maps were created using the software program SURFER (Golden Software 2002), creating a visual indication of contamination levels for each plot. In addition to quantifying heavy metal concentrations, other soil properties such as pH, organic matter, Particle Size Distribution and Total Nitrogen (Chapter 3) are presented alongside contamination data.

Data tables also compare the contamination results to guideline figures discussed earlier in the chapter, showing the number and percentage of individual samples exceeding the relevant guideline values. In the case of CLEA guidelines, consideration is also given to the mean contaminant concentration and if this falls below the Soil Guideline value (SGV) as a way of averaging exposure. Due to variation and uncertainties with the mean a direct comparison could be misleading. Statistical tests can help improve the reliability of soil sampling techniques (CL:AIRE 2004). The CLEA publication CLR 7 recommends comparing the upper 95th percentile with the SGV. If the calculated upper 95th percentile (US95) is less than the soil SGV then contamination levels can be considered to fall below the relevant guideline (DEFRA and Environment Agency 2002a). For all elements with the exception of Pb the US95 is based on arithmetic mean, for Pb the geometric mean is used due to the way lead is modeled within the CLEA framework. This test should, however, be treated with caution and high point sources or other areas may require further investigation, such as maximum value tests also provided in CLR 7.

The results of soil contamination have been compared to CLEA SGV values as well as ICRCL phytotoxic, or when no phytotoxic level is given, to allotments / domestic gardens, as the most sensitive land use. Even though ICRCL values have officially been withdrawn these were the original guidelines in place when work commenced on this project, and remain the only published guidelines in relations to phytotoxicity. The CLEA values for gardens / allotments have been used for the reasons described above, although in several cases this seldom differs from the residential land use.

One significant use of the contamination maps was to define areas of the plot with consistent elevated contaminant levels, thus creating hotspots in which to carry out more detailed monitoring of soil / plant interactions. Using the maps, genuinely elevated areas were defined and amalgamated to consider all elements, as shown in figure 4.10.1. This process was partly subjective, but as far as practicable covered all elements and in many cases hot spots were synergistic, indicating patches of waste material or similar. From within these areas a range of tree species were selected that, (i), fell within the hotspot area and adjacent to a soil sampling point and, (ii), reflected all species in equal measure across the plot. These were final experimental units, and have been defined by the red and blue circles in the example figures 4.5.3 and 4.4.2. Red circles received a full compliment of monitoring over 3 years, the blue areas were monitored at a lesser frequency. Details of this monitoring are given in Chapter 5.

The data are presented by site and then by individual element. Scales have been standardised for each element. For each map the sample code is printed above the line and the actual element concentration for each point given below the sample point symbol in mg kg^{-1}. Where sufficient data exists maps will also show pH variation across the plot.

4.4.1 Cromdale Grove 1 contamination distribution

Table 4.2. Total soil metal concentrations (mg/kg) and physiochemical parameters at Cromdale Grove 1 (CRM1) at beginning of project (Year 1)

CRM 1		As	Cd	Cu	Ni	Pb	Zn
Number			47	71	71	71	71
MIN			0.0	10.4	10.1	62.2	56.2
MAX			1.0	883.5	109.4	560.1	826.6
Arth. Mean			0.3	124.4	57.8	215.4	298.1
Geo. Mean			0.3	94.4	50.2	198.3	253.9
Median			0.3	90.4	63.5	208.5	248.7
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	1	n/a	50	450*	n/a
No > ICRCL			0	19	27	2	27
No > CLEA			0	n/a	44	No	n/a
% > ICRCL			0%	27%	38%	3%	38%
% > CLEA			0%	n/a	62%	No	n/a
Mean > SGV			No	n/a	**Yes**	No*	n/a
US95 < SGV			PASS	n/a	**FAIL**	PASS*	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
CRM 1	23	4.88	7.67	6.38	6.83

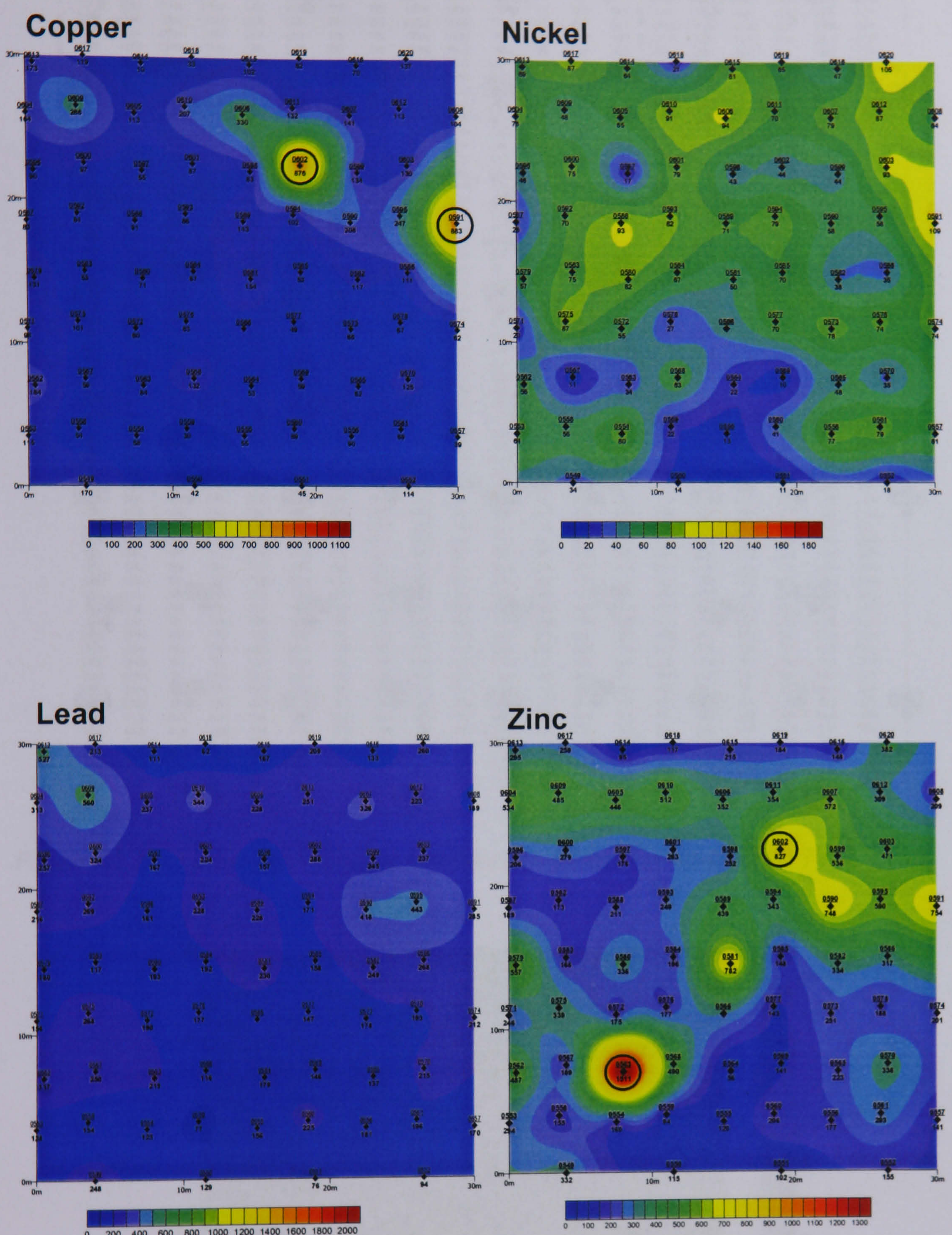
Organic Matter
LOI %
11.15

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
CRM 1	13.1	28.9	58.0	Sandy silt loam

Total Nitrogen
%N
0.280

Cromdale Grove is predominantly contaminated with Cu, Ni and Zn above ICRCL phytotoxic levels. Cu and Zn show several high values and similar hotspot dispersion. Soil pH is highly variable across the plot, possibly caused by patches of poorly incorporated colliery spoil. The site has relatively high total nitrogen, probably due to a combination of local atmospheric NO_x deposition, fertiliser application and dog excrement.

Figure 4.2.1. Contamination maps for Cromdale Grove



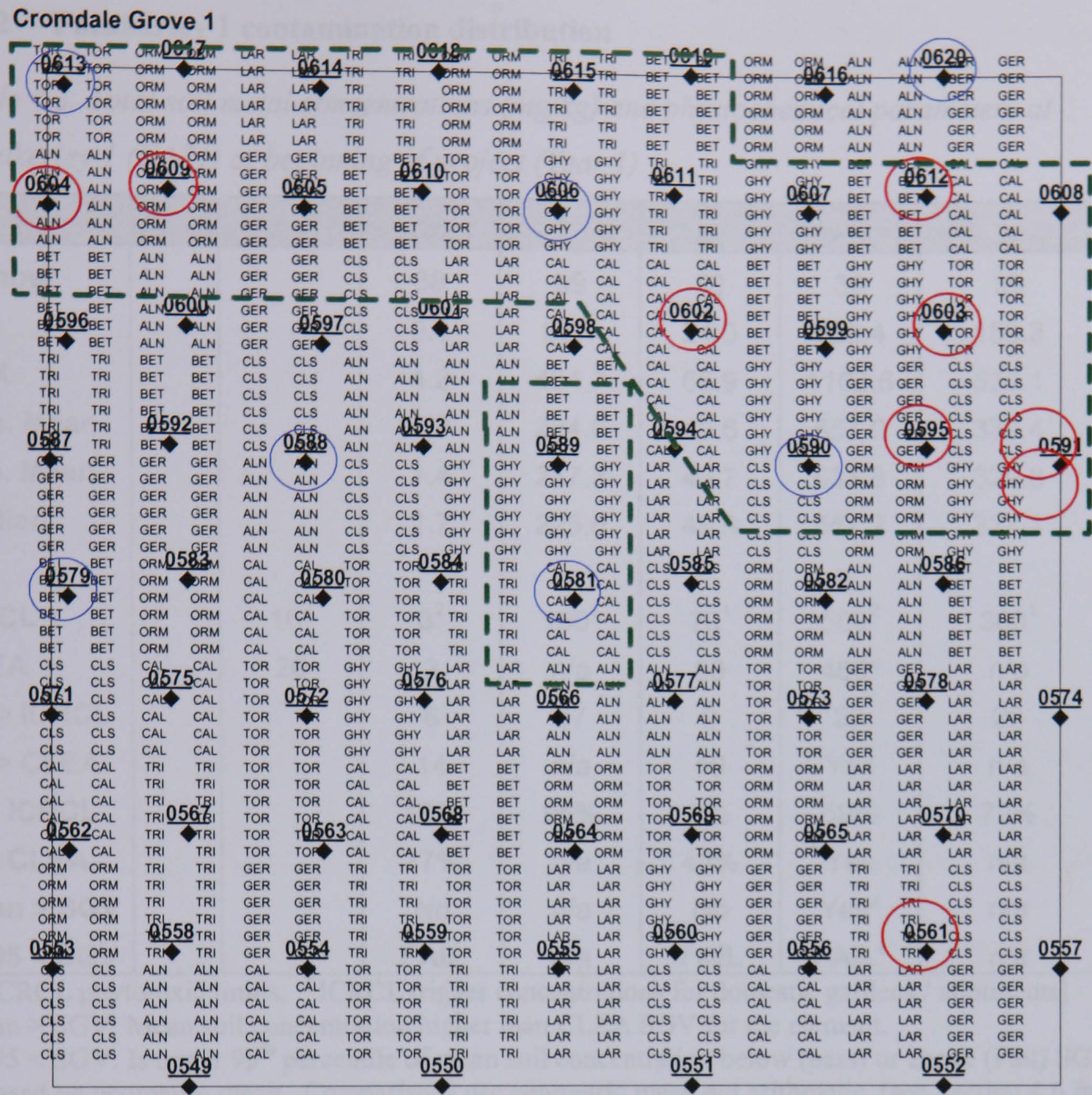


Figure 4.2.2. Hotspot definition and intensive experimental areas on CRM 1

Figure 4.2.3. Soil pH map of CRM1

4.4.2 Fazakerley 1 contamination distribution

Table 4.3. Total soil metal concentrations (mg/kg) and physiochemical parameters at Fazakerley 1 (FAZ1) at beginning of project (Year 1)

FAZ 1		As	Cd	Cu	Ni	Pb	Zn
Number			38	39	39	39	39
MIN			0.1	95.1	24.0	228.4	159.3
MAX			4.2	404.7	66.9	1102.6	536.1
Arth. Mean			1.8	224.0	49.6	557.0	338.4
Geo. Mean			1.4	217.3	48.7	533.8	329.8
Median			1.7	225.6	49.9	557.2	327.1
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	2	n/a	50	450*	n/a
No > ICRCL			6	37	0	23	28
No > CLEA			14	n/a	19	Yes	n/a
% > ICRCL			16%	95%	0%	59%	72%
% > CLEA			37%	n/a	49%	Yes	n/a
Mean > SGV			No	n/a	No	Yes*	n/a
US95 < SGV			FAIL	n/a	FAIL	FAIL*	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
FAZ 1	21	7.07	8.12	7.59	7.31

Organic Matter
LOI %
6.88

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
FAZ 1	16.3	19.6	64.1	Silt loam

Total Nitrogen
%N
0.225

Faz 1 shows contamination from all elements tested, particularly Cu, Pb and Zn. Broadly speaking these elements are dispersed diffusely with no easily recognisable hotspots. Several high point sources are shown for Pb, but these are inconsistent with the surrounding elevated areas. The location of this plot within five metres of a busy urban road may account for a high, diffuse level of lead. Other elements may be present historically in sewage sludge products that have been dumped on the site over a long time period, becoming well mixed, especially due to recent landscaping at the site.

Figure 4.3.1. Contamination maps for FAZ1

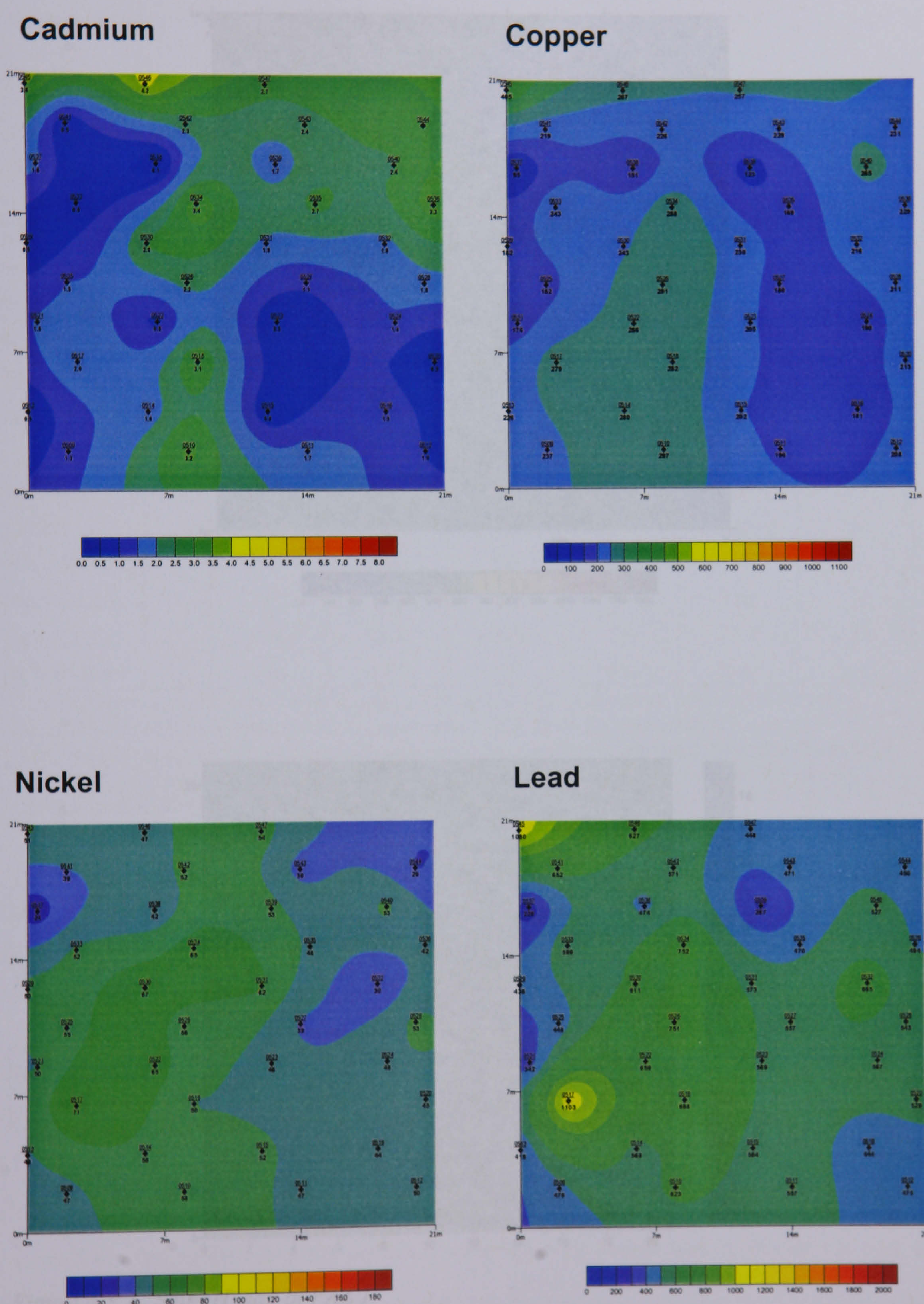
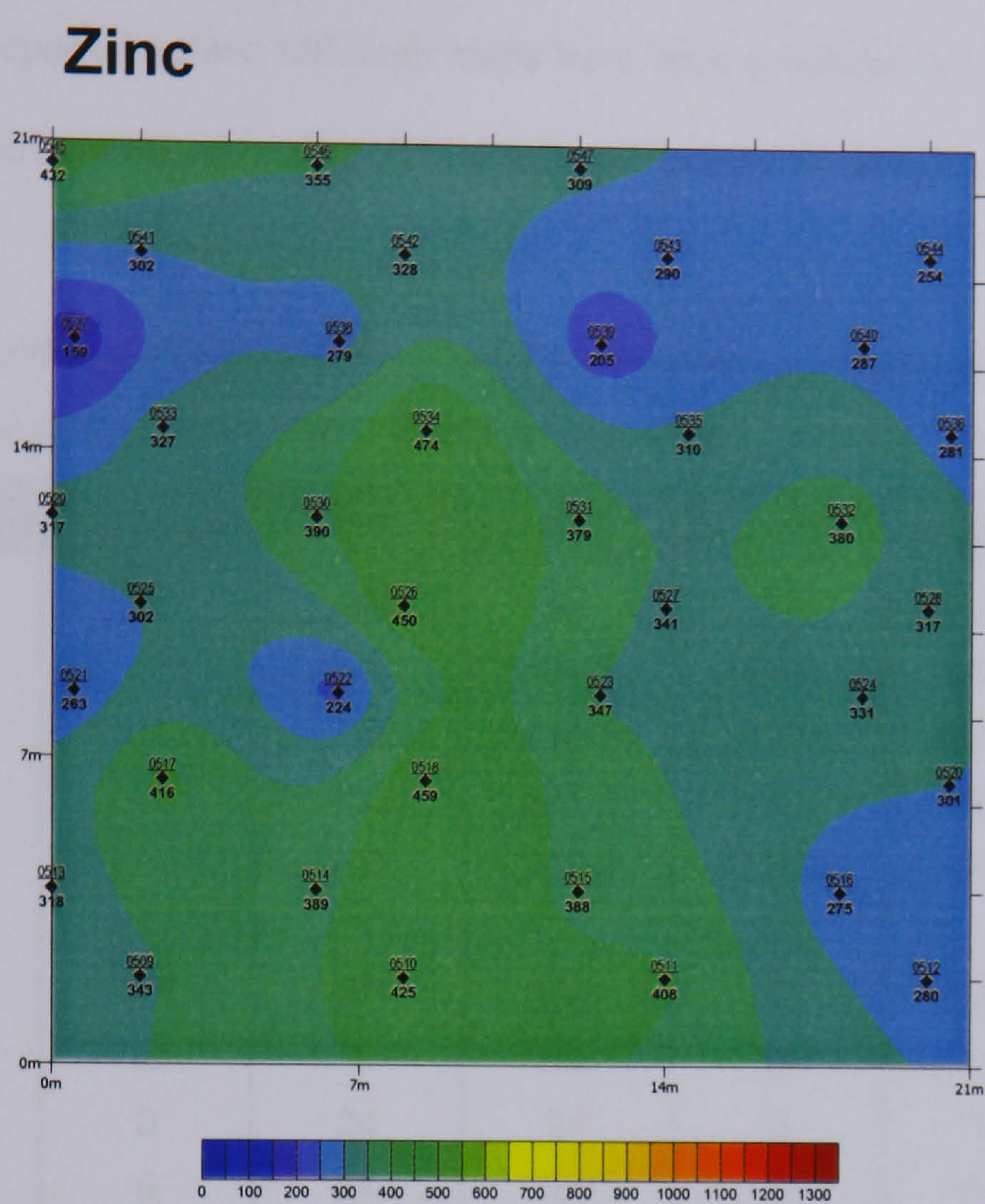


Figure 4.3.1. Contamination maps for FAZ1 (con't)



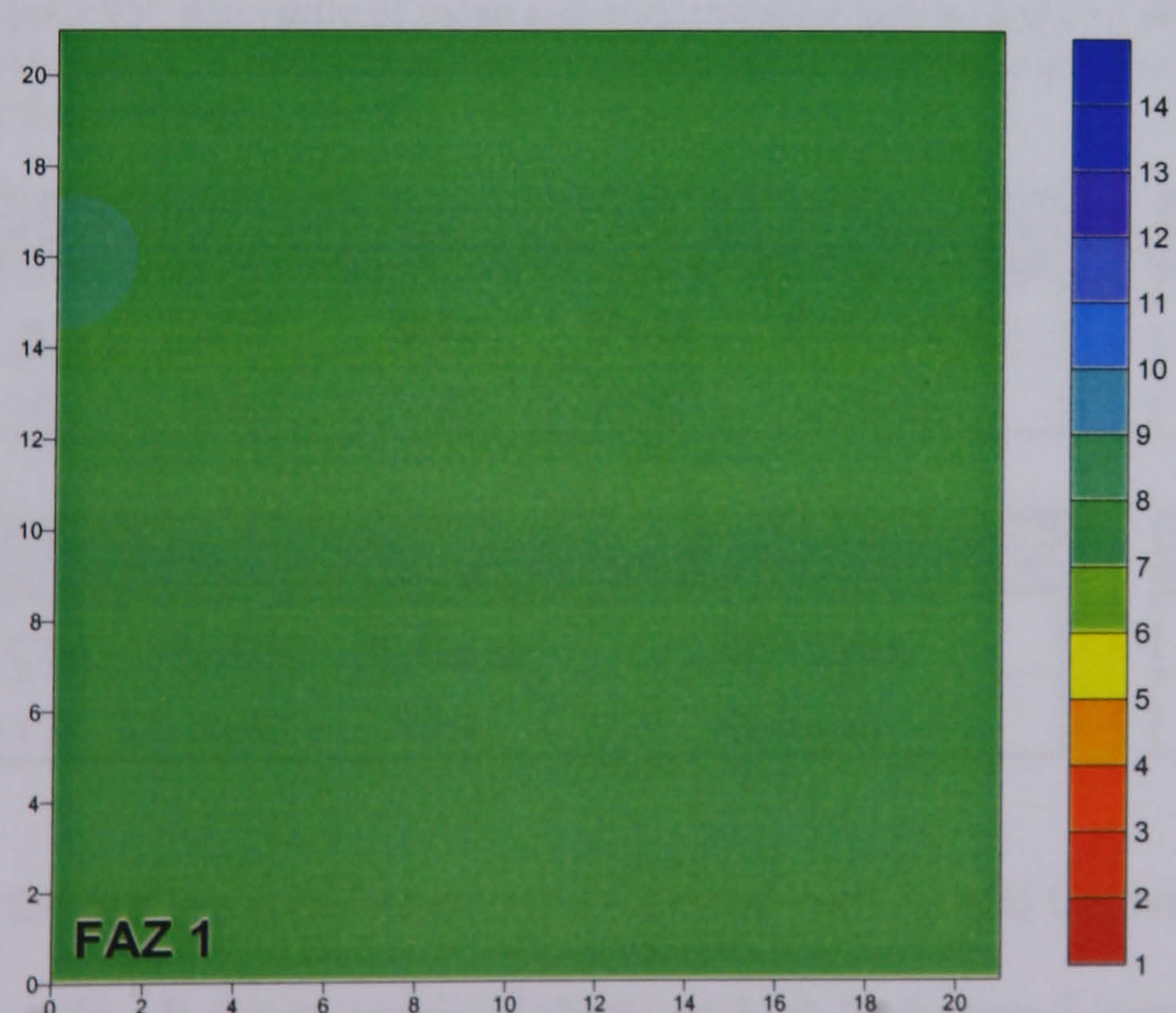


Figure 4.3.2. Soil pH map of FAZ 1

4.4.3 Fazakerley 2 contamination distribution

Due to different experimental treatments on the FAZ 2 plot, each treatment is considered separately when reported below, although maps have been produced for the entire plot with individual treatments indicated. For full details of treatment design and layout refer to Chapter 3.

Table 4.4. Total soil metal concentrations (mg/kg) and physiochemical parameters at Fazakerley 2 Control Treatment (FAZ2 CON) at beginning of project (Year 1)

FAZ 2 (CON)		As	Cd	Cu	Ni	Pb	Zn
Number			24	24	24	24	24
MIN			3.7	144.4	24.7	249.5	108.5
MAX			7.9	399.0	47.1	624.3	331.0
Mean			6.1	307.7	34.9	463.9	251.1
Median			6.3	320.3	33.3	469.7	265.3
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	1	n/a	50	450 (avg)	n/a
No > ICRCL		0	24	24	0	10	5
No > CLEA		0	24	n/a	0	Yes	n/a
% > ICRCL		0%	100%	100%	0%	42%	21%
% > CLEA		0%	100%	n/a	0%	Yes	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
FAZ 2 CON	10	5.94	7.49	6.57	6.86

Organic Matter
LOI %
6.91

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
FAZ 2 CON	11.2	13.9	74.9	Silt loam

Total Nitrogen
%N
0.226

Control areas of the plot are significantly contaminated with Cd and Cu, with some lead and a small amount of Zn. It is not certain if these are high background levels for the control areas (eg: from previous sludge dumping), or if cross contamination has occurred from neighboring treatments. Soil pH is slightly more neutral than in the other treatments.

Table 4.4.1. Total soil metal concentrations (mg/kg) and physiochemical parameters at Fazakerley 2 Sewage Cake Treatment (FAZ2 CAK) at beginning of project (Year 1)

FAZ 2 (CAK)		**As**	**Cd**	**Cu**	**Ni**	**Pb**	**Zn**
Number			18	18	18	18	18
MIN			4.1	147.5	19.8	157.6	104.2
MAX			7.1	408.9	50.2	564.3	463.1
Mean			5.7	273.0	34.4	393.5	228.8
Median			5.7	274.2	33.9	436.2	236.4
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	1	n/a	50	450 (avg)	n/a
No > ICRCL			18	18	0	1	1
No > CLEA			18	n/a	1	No	n/a
% > ICRCL			100%	100%	0%	6%	6%
% > CLEA			100%	n/a	6%	No	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95^{th} percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_20)				
Site	**n**	**MIN**	**MAX**	**Mean**	**Bulk Samp**
FAZ 2 CAK	7	5.70	7.05	6.37	6.25

Organic Matter
LOI %
7.15

	Soil Particle Size Distribution			
Site	**% Clay**	**% Silt**	**% Sand**	**Soil Type**
FAZ 2 CAK	nt	nt	nt	

Total Nitrogen
%N
0.260

Table 4.4.2. Total soil metal concentrations (mg/kg) and physiochemical parameters at Fazakerley 2 Sewage Sludge Treatment (FAZ2 SLU) at beginning of project (Year 1)

FAZ 2 (SLU)		As	Cd	Cu	Ni	Pb	Zn
Number			17	17	17	17	17
Min			5.0	51.5	23.6	304.8	202.5
Max			7.7	373.0	178.5	555.3	312.7
MEAN			6.0	282.1	42.6	421.2	272.0
Median			6.0	300.0	34.8	409.0	274.5
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	1	n/a	50	450 (avg)	n/a
No > ICRCL		1	17	16	1	2	5
No > CLEA		0	17	n/a	1	No	n/a
% > ICRCL		0%	100%	94%	6%	12%	29%
% > CLEA		0%	100%	n/a	6%	No	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
FAZ 2 SLU	6	5.11	7.23	6.12	6.02

Organic Matter
LOI %
nt

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
FAZ 2 SLU	nt	nt	nt	

Total Nitrogen
%N
0.282

Figure 4.4.1. Contamination maps for FAZ2

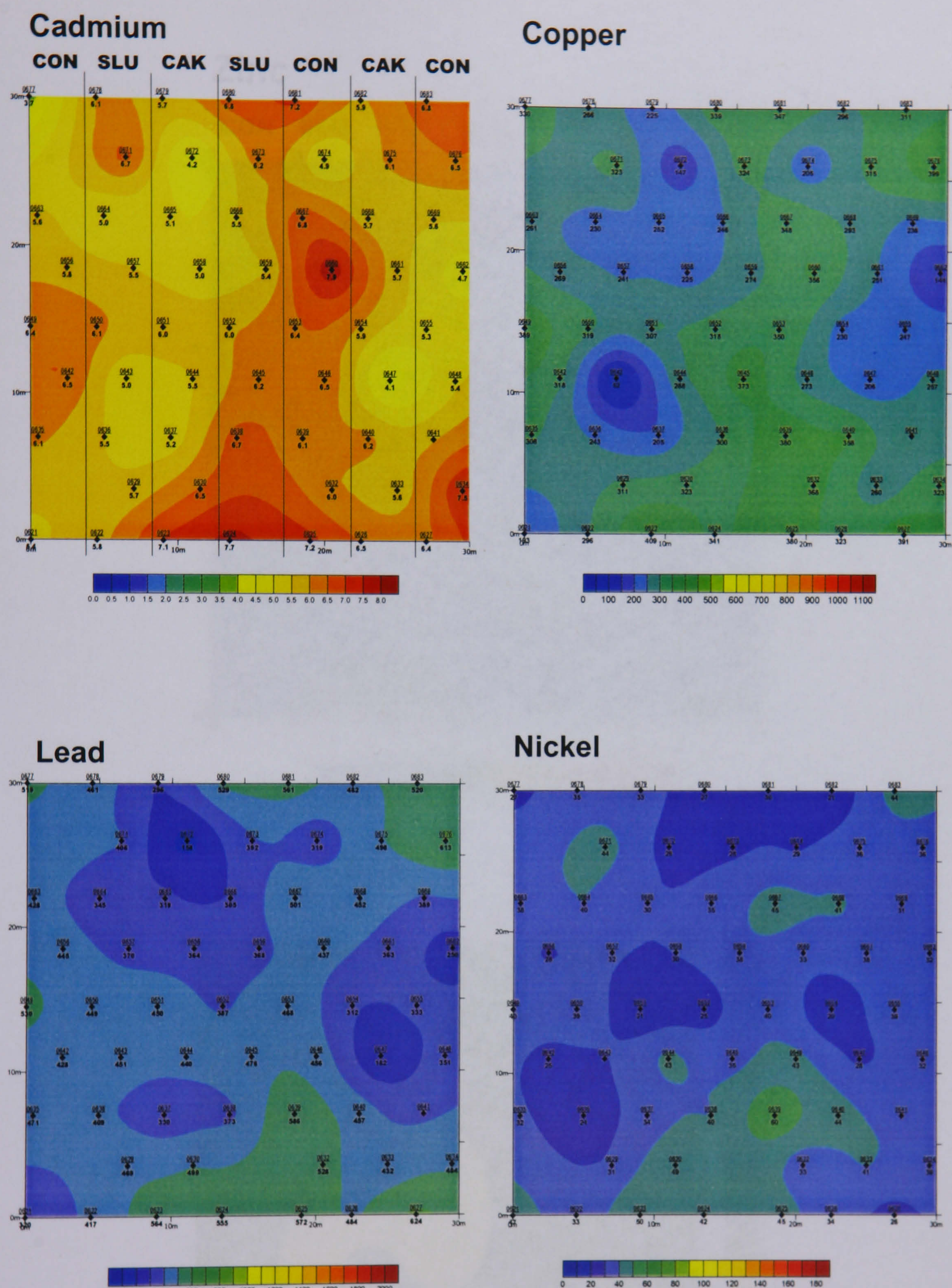


Figure 4.4.1. Contamination maps for FAZ2 (con't)

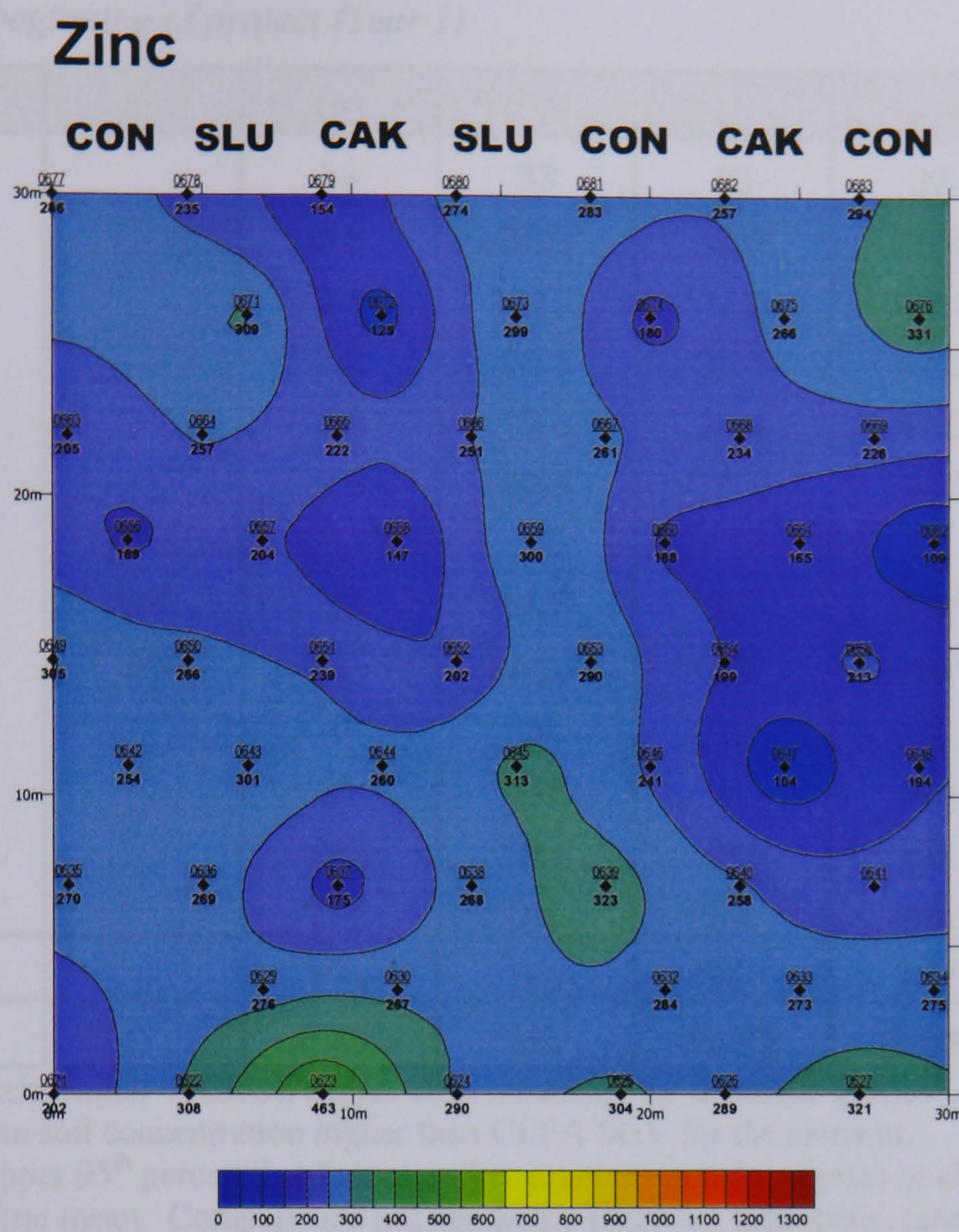


Figure 4.4.2. Soil pH map of FAZ 2

4.4.4 Kirby Moss contamination distribution

Table 4.5. Total soil metal concentrations (mg/kg) and physiochemical parameters at Kirby Moss (KIR1) at beginning of project (Year 1)

KIR 1		As	Cd	Cu	Ni	Pb	Zn
Number			33	33	33	32	33
MIN			0.2	32.0	12.2	16.8	61.9
MAX			3.4	177.3	37.8	169.0	199.3
Arth. Mean			2.2	86.3	25.2	49.9	100.2
Geo. Mean			2.0	79.2	24.4	44.5	93.9
Median			2.3	82.5	25.7	44.0	85.7
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	1	n/a	50	450*	n/a
No > ICRCL			6	5	0	0	0
No > CLEA			31	n/a	0	No	n/a
% > ICRCL			18%	15%	0%	0%	0%
% > CLEA			94%	0%	0%	n/a	n/a
Mean > SGV			Yes	n/a	No	No*	n/a
US95 < SGV			FAIL	n/a	PASS	PASS*	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
KIR 1	14	5.50	6.68	6.23	6.35

Organic Matter
LOI %
5.56

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
KIR 1	7.5	9.7	82.8	Silt Loam

Total Nitrogen
%N
0.135

Cd and Cu are slightly elevated in KIR 1, all other elements are low. Although 94% of Cd results are higher than the CLEA SGV (which is low due to the sites low pH), this is only a 2 mg kg^{-1} decrease from the ICRCL limits but makes a large impact on the way the site is perceived. Due to Kirby Mosses remote location it is unlikely to have as much access as other urban sites, and the SGV's quoted here would undoubtedly allow an increase if a different land use were adopted.

Figure 4.5.1. Contamination maps for KIR 1

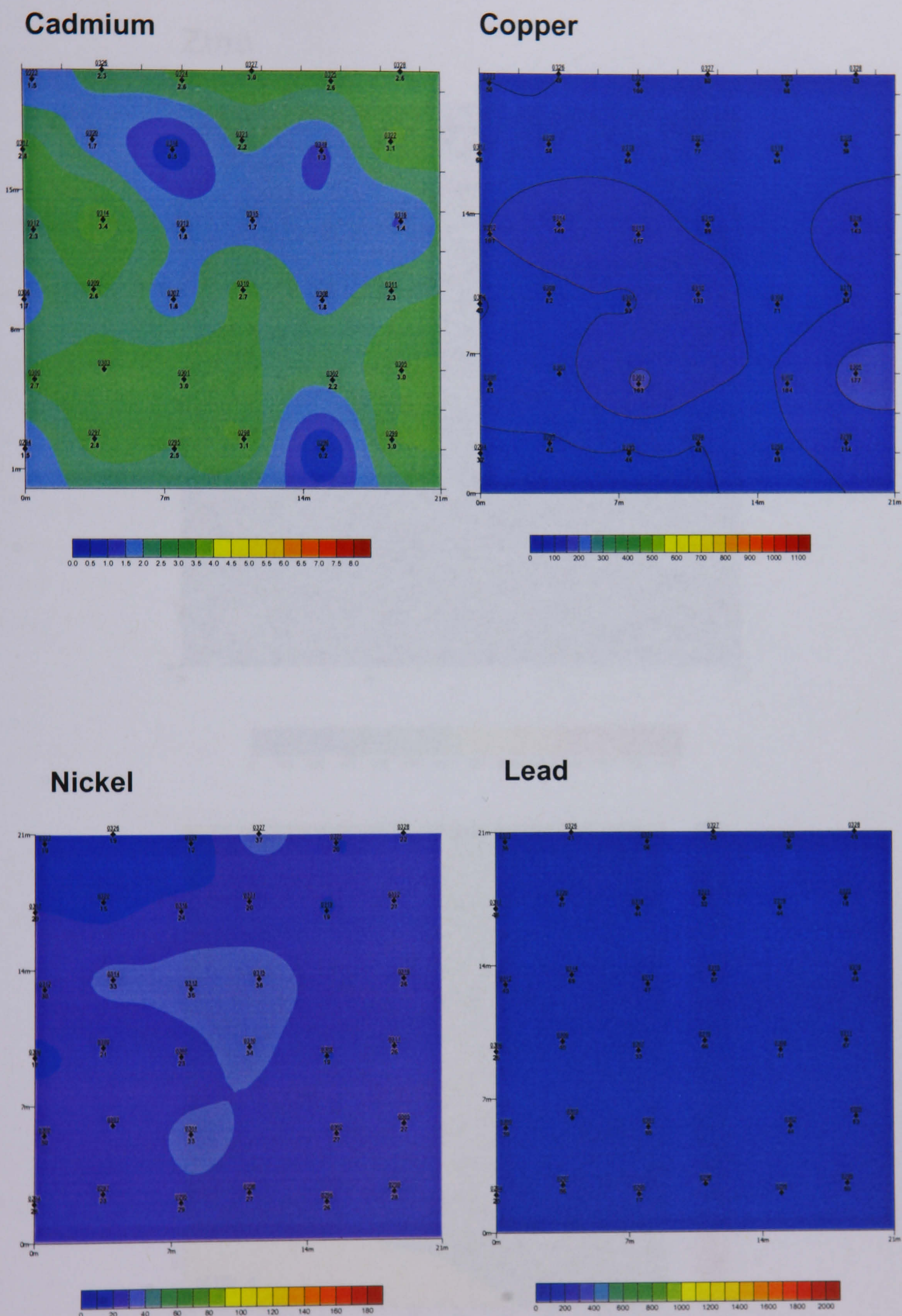
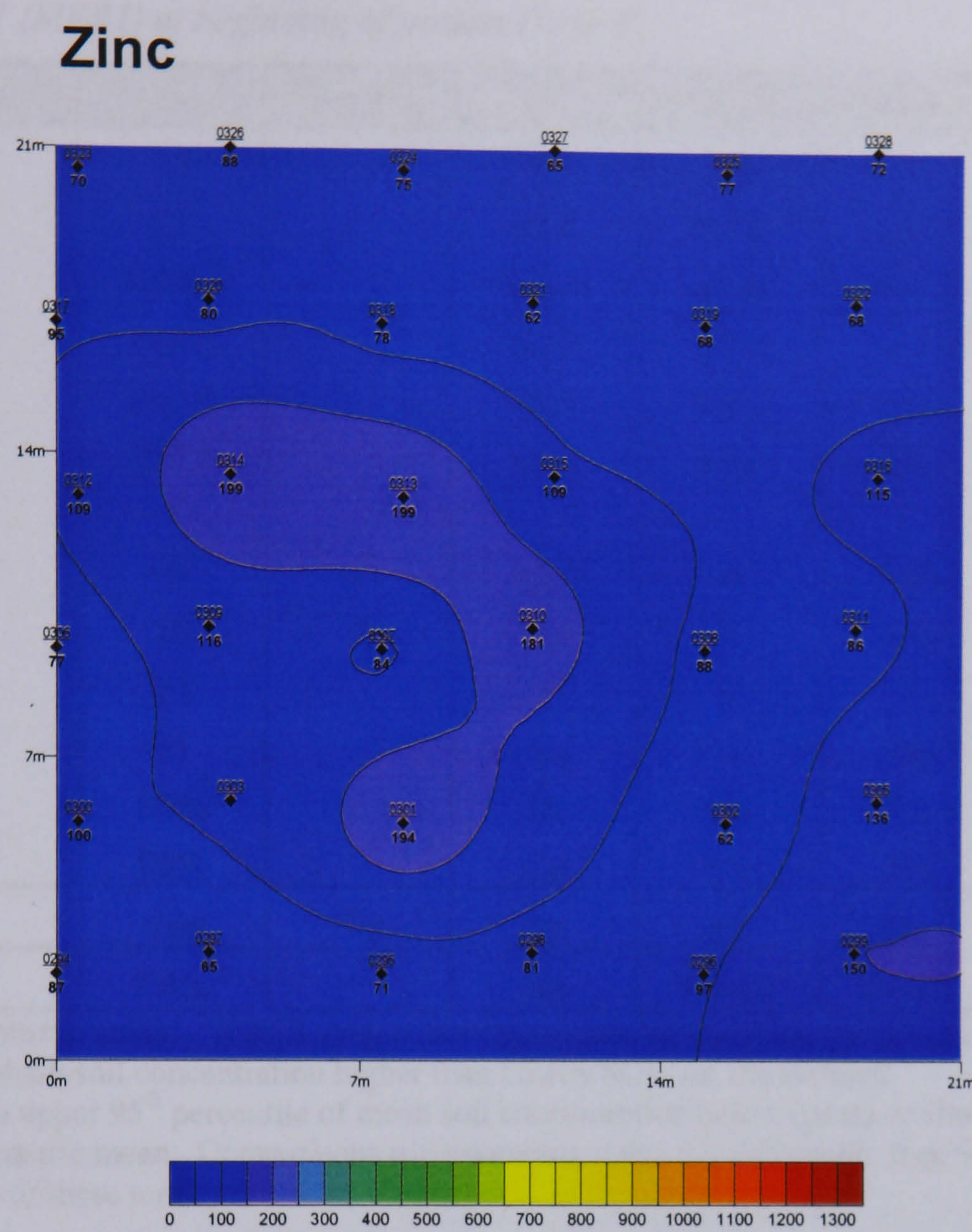


Figure 4.5.1. Contamination maps for KIR 1 (cont)



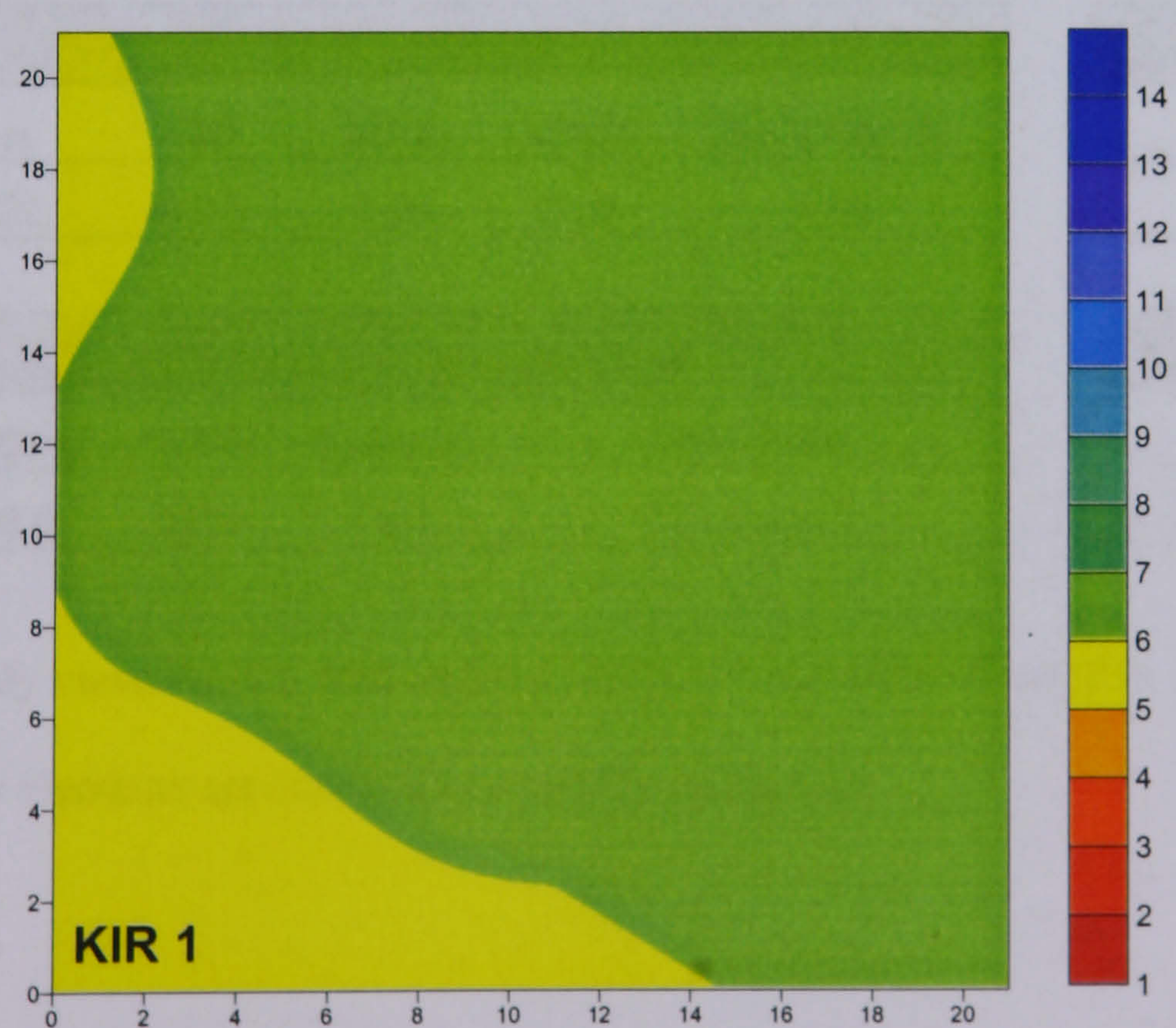


Figure 4.5.2. Soil pH map of KIR 1

4.4.5 Merton Bank 1 contamination distribution

Table 4.6. Total soil metal concentrations (mg/kg) and physiochemical parameters at Merton Bank 1 (MER1) at beginning of project (Year 1)

MER 1		As	Cd	Cu	Ni	Pb	Zn
Number		72		72	72	71	71
MIN		5.1		23.4	22.3	38.6	27.8
MAX		5265.6		144.8	41.4	394.7	339.2
Arth. Mean		642.0		62.6	32.7	117.5	135.5
Geo. Mean		311.6		58.7	32.4	99.0	120.7
Median		297.7		55.0	33.2	98.2	114.1
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	2	n/a	50	450*	n/a
No > ICRCL		71		2	0	0	1
No > CLEA		71		n/a	0	No	n/a
% > ICRCL		99%		3%	0%	0%	1%
% > CLEA		99%		n/a	0%	No	n/a
Mean > SGV		Yes	N/a	n/a	No	No*	n/a
US95 < SGV		FAIL		n/a	PASS	PASS*	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
MER 1	22	6.95	7.80	7.32	7.35

Organic Matter
LOI %
8.75

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
MER 1	19.8	32.0	48.3	Clay Loam

Total Nitrogen
%N
0.241

Arsenic is highly elevated in a well defined hotspot at one edge of the plot, at the top of the bank. No other elements are elevated in a significant manner.

Figure 4.6.1. Contamination maps for MER 1

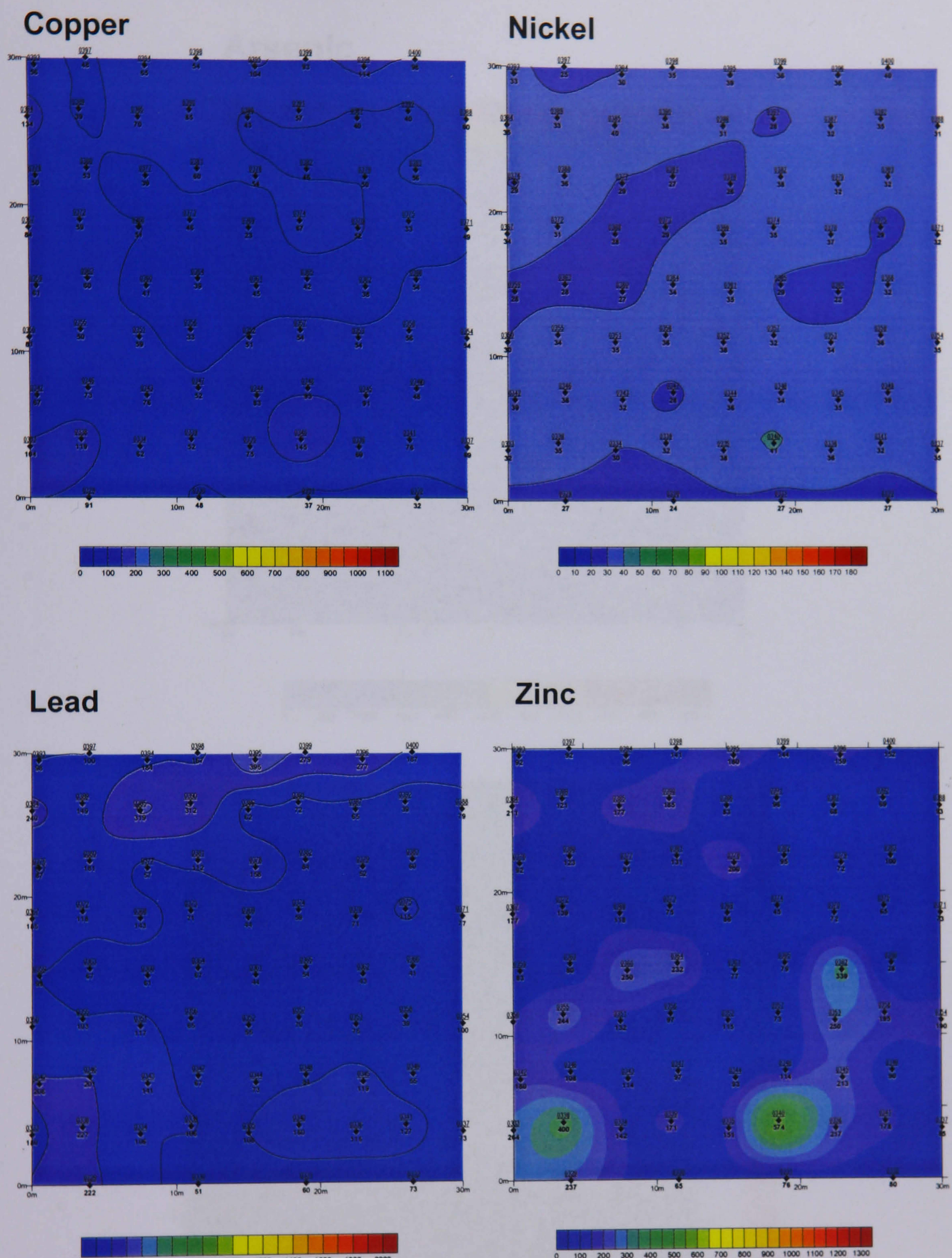
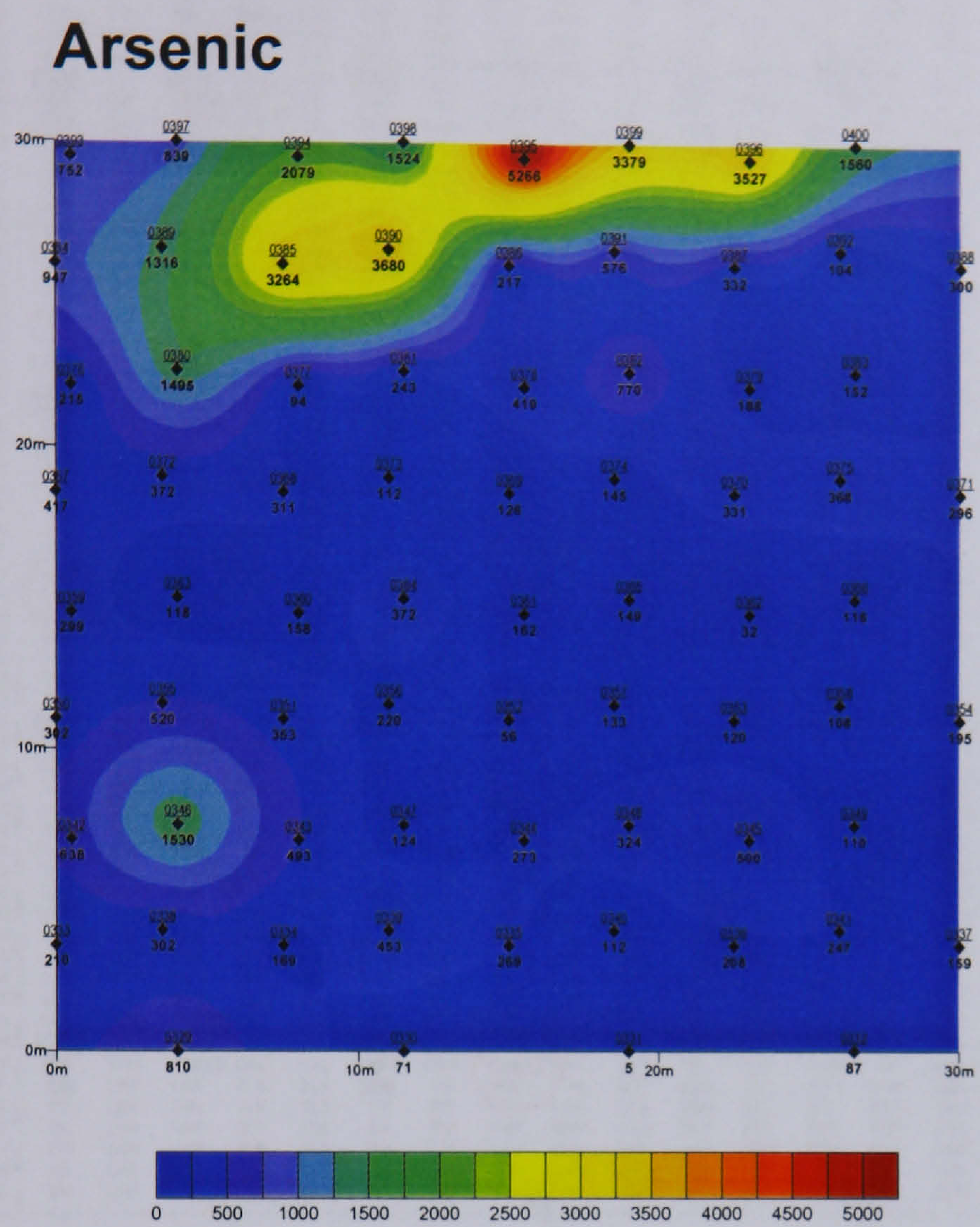


Figure 4.6.1. Contamination maps for MER 1 (cont'd)



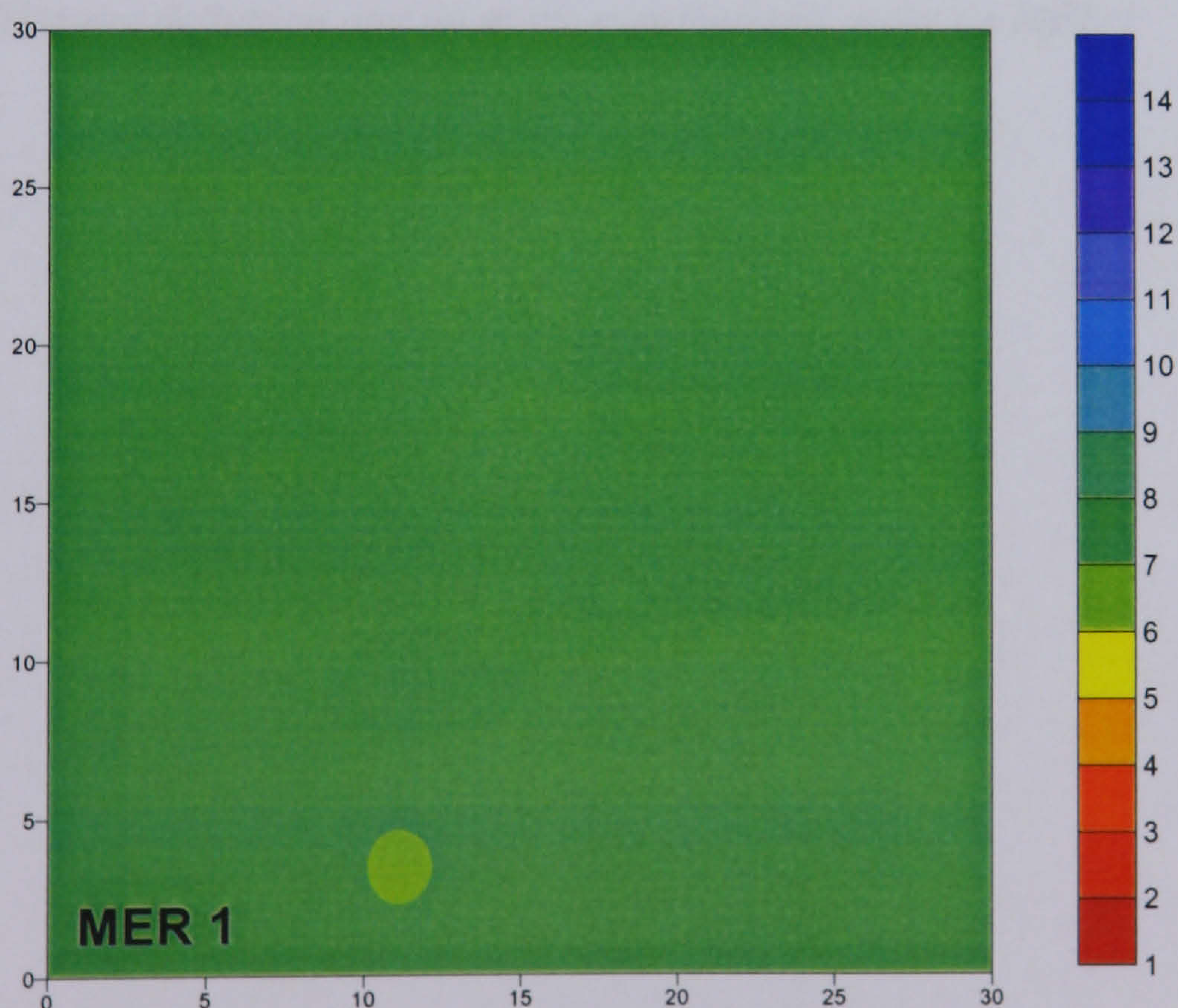


Figure 4.6.2. Soil pH map of MER 1

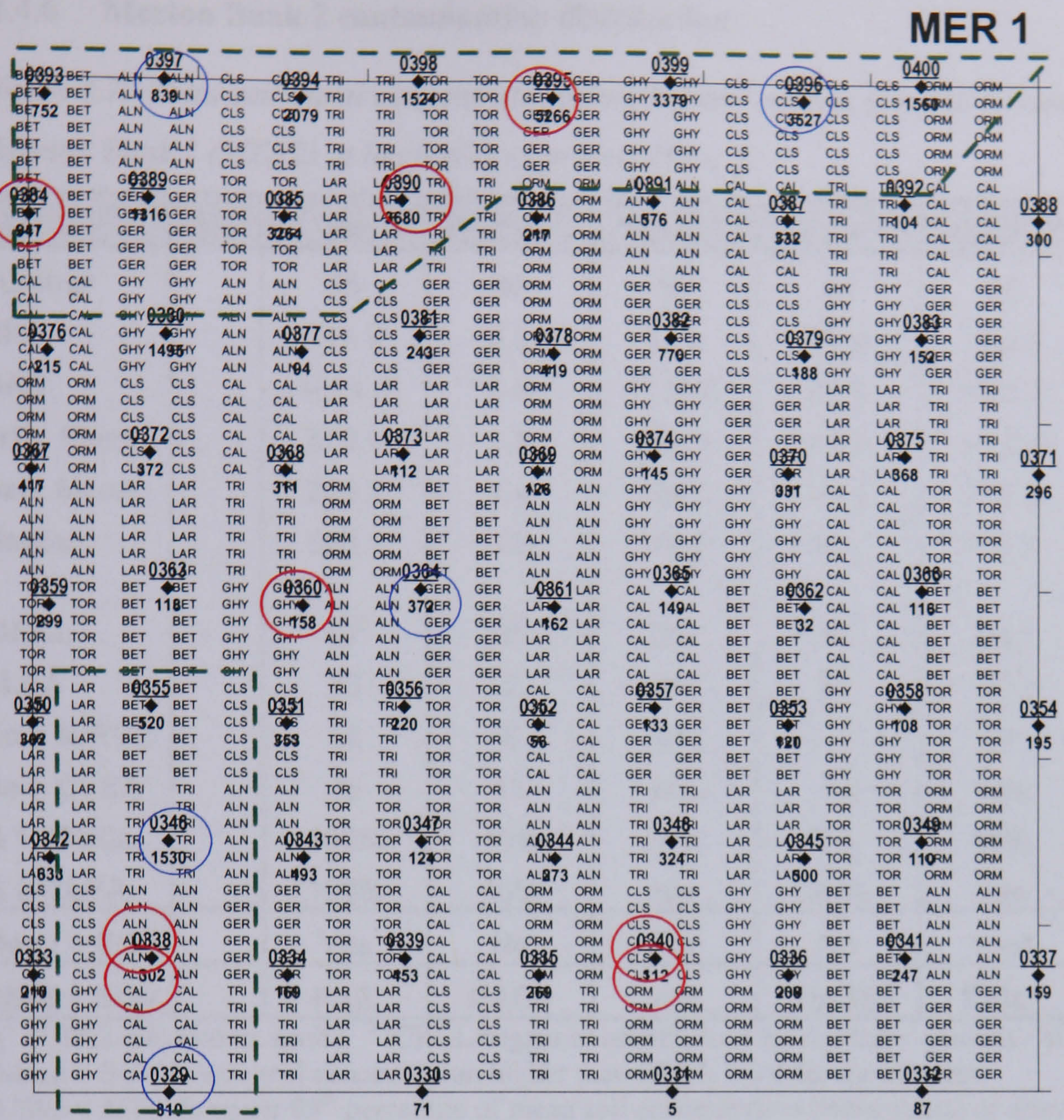


Figure 4.6.3. Hotspot definition and intensive experimental areas on MER 1

4.4.6 Merton Bank 2 contamination distribution

Table 4.7. Total soil metal concentrations (mg/kg) and physiochemical parameters at Merton Bank 2 (MER2) at beginning of project (Year 1)

MER 2		As	Cd	Cu	Ni	Pb	Zn
Number		36	36	36	36	36	36
MIN		114.9	0.0	55.2	25.9	120.5	102.9
MAX		4611.9	4.5	1146.5	62.8	3641.5	729.8
Arth. Mean		878.6	1.8	280.1	46.3	1116.9	248.2
Geo. Mean		615.3	1.4	194.0	45.4	719.6	212.4
Median		638.1	1.8	176.0	44.4	710.6	181.1
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	2	n/a	50	450*	n/a
No > ICRCL		36	5	21	0	21	10
No > CLEA		36	13	n/a	13	Yes	n/a
% > ICRCL		100%	14%	58%	0%	58%	28%
% > CLEA		100%	36%	n/a	36%	Yes	n/a
Mean > SGV		Yes	No	n/a	No	Yes*	n/a
US95 < SGV		FAIL	PASS	n/a	PASS	FAIL*	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
MER 2	17	6.99	7.78	7.48	7.43

Organic Matter
LOI %
13.11

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
MER 2	21.1	37.2	41.7	Clay loam

Total Nitrogen
%N
0.298

Consistent with all Merton Bank sites, As is highly elevated on this plot, with 100% of samples over guidelines, and a maximum concentration over 230 times the SGV limits. Lead and copper show well defined elevated areas, and to a lesser extent so does Zinc. These four elements show similar geography in location of the hotspots, showing the elements appear to be present together. Cadmium and Nickel are present in relatively very small amounts, although the application of different guidelines (i.e.: CLEA over ICRCL) can alter significantly the percentage of sampling points exceeding guidance limits.

Figure 4.7.1. Contamination maps for MER 2

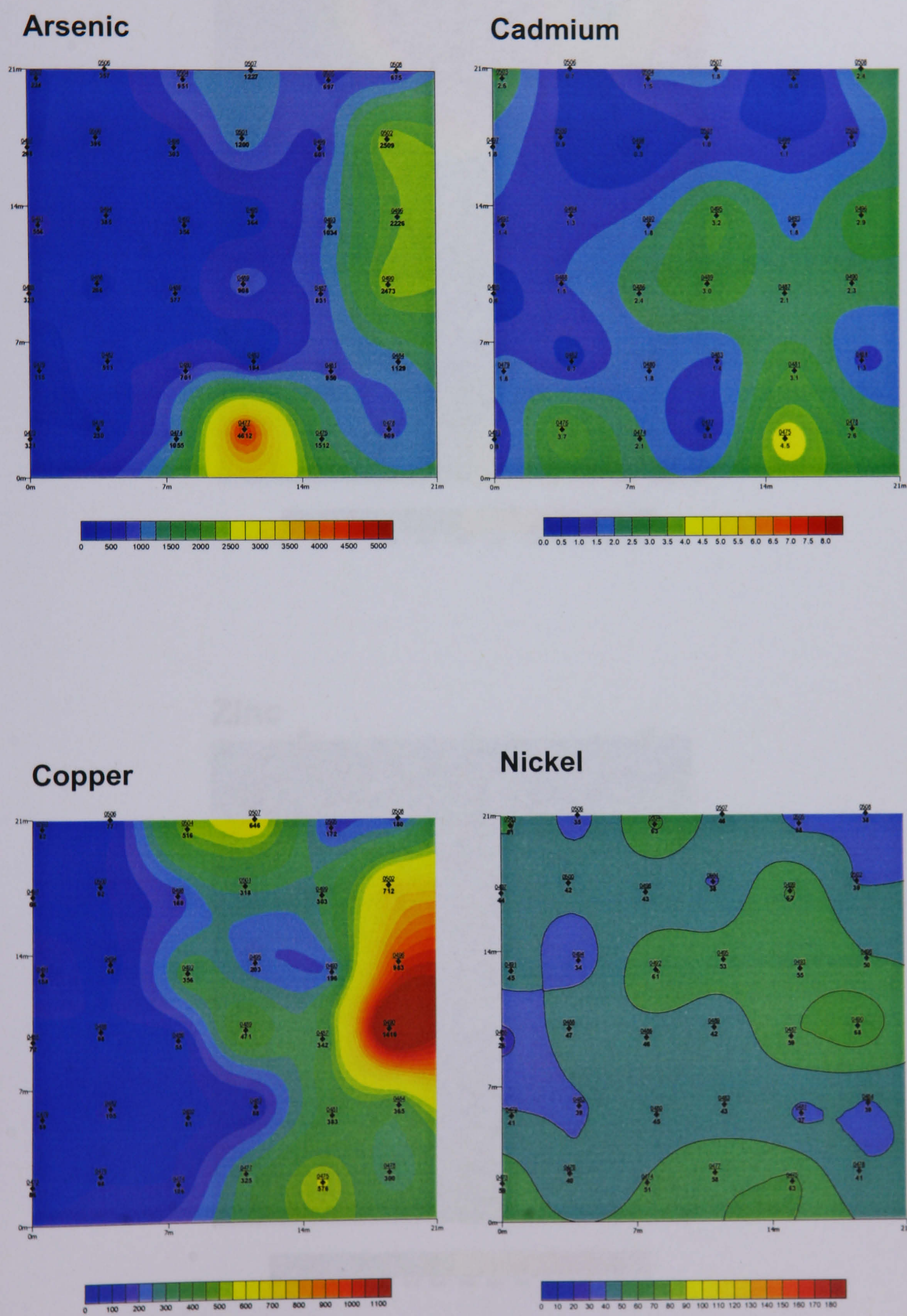
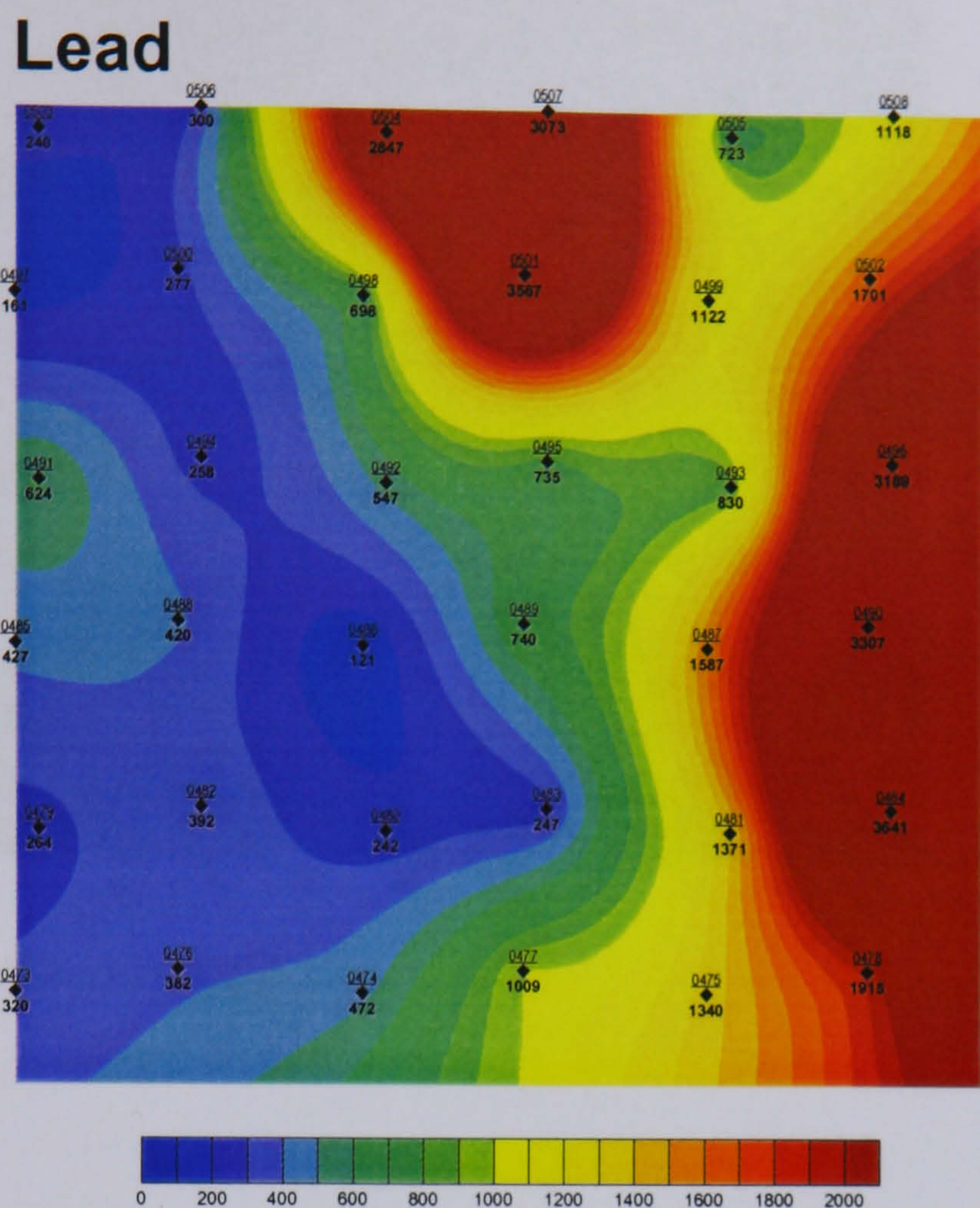
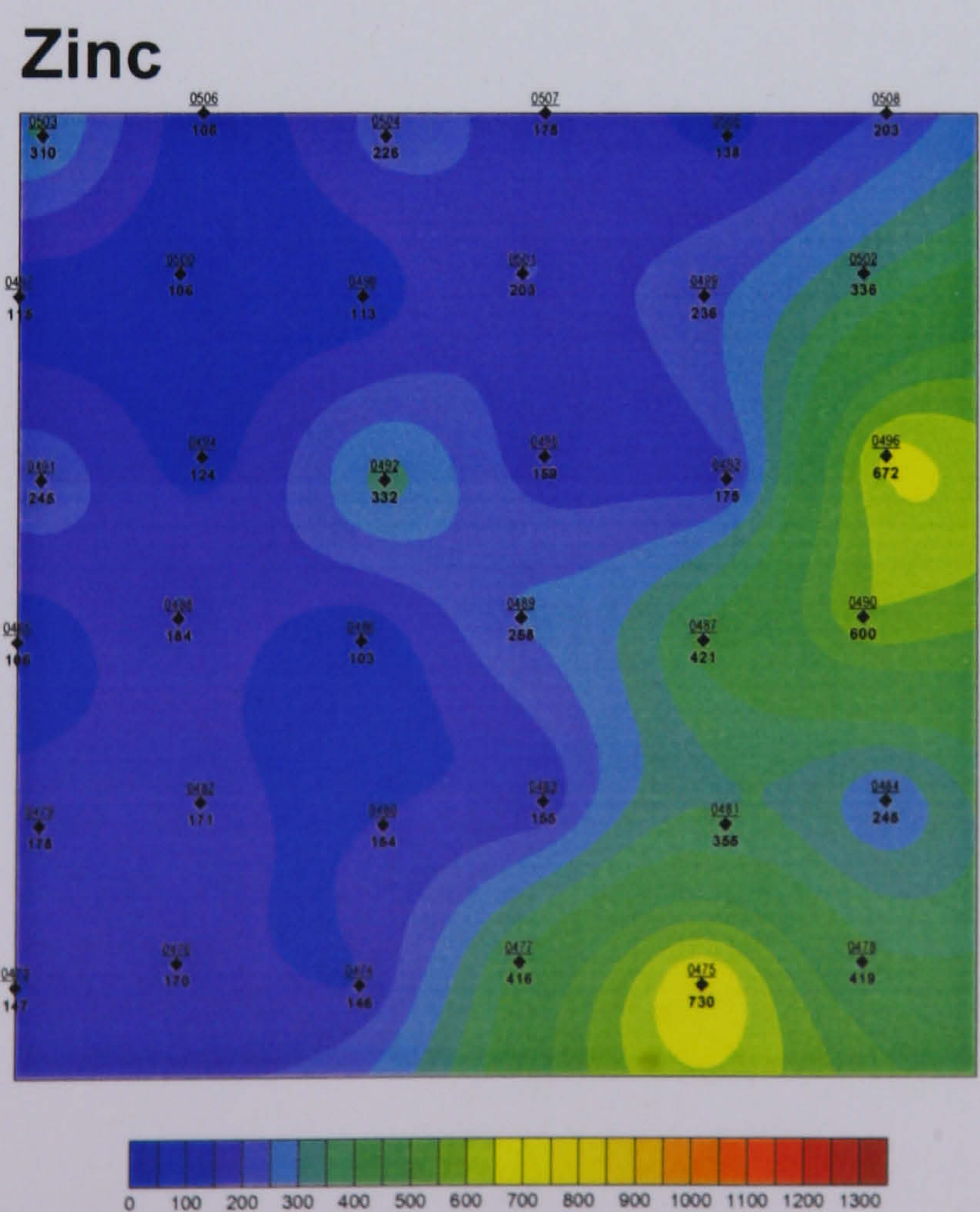


Figure 4.7.1. Contamination maps for MER 2 (cont'd)





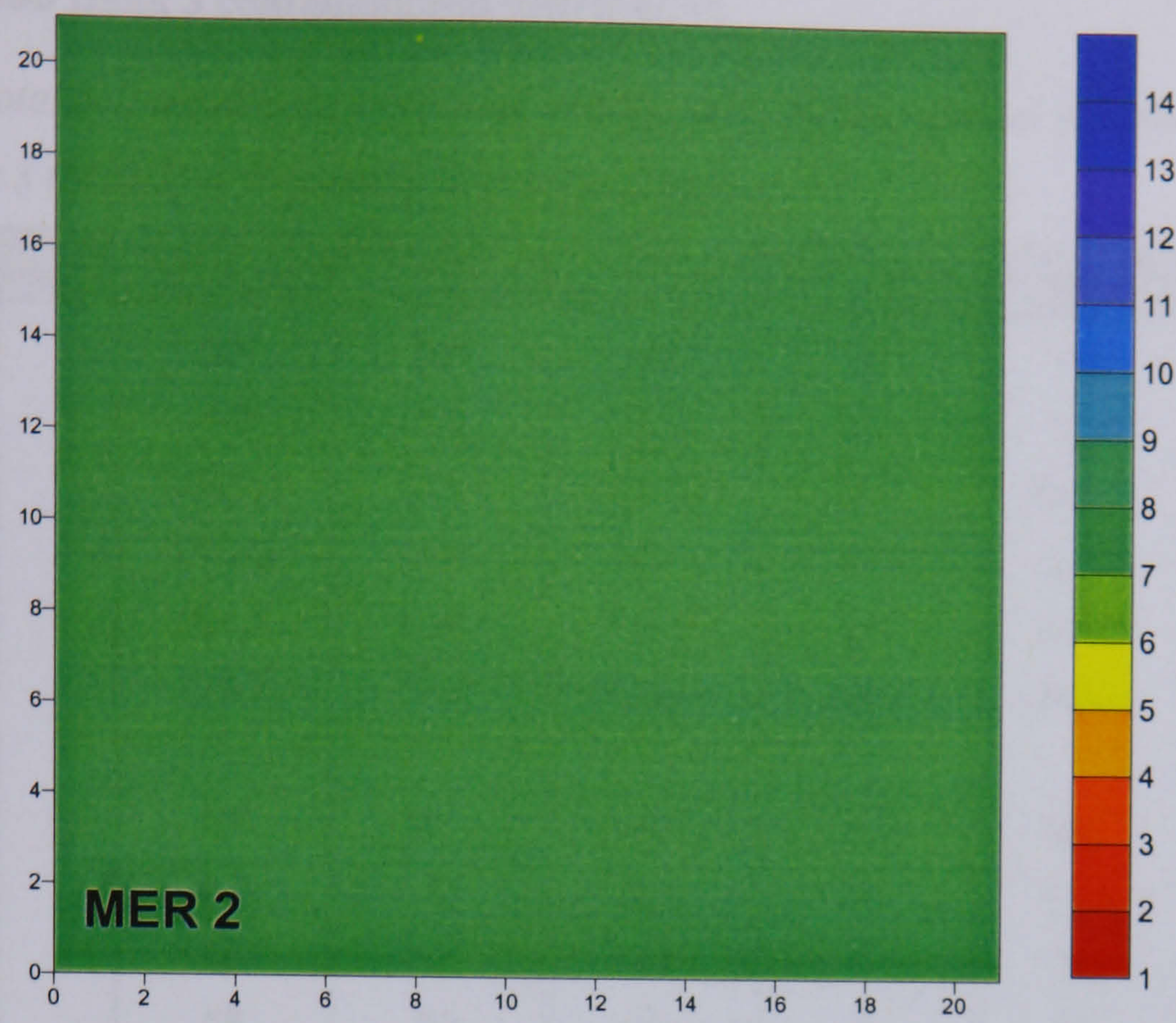


Figure 4.7.2. Soil pH map of MER 2

4.4.7 Merton Bank 3 contamination distribution

Table 4.8. Total soil metal concentrations (mg/kg) and physiochemical parameters at Merton Bank 3 (MER3) at beginning of project (Year 1)

MER 3		As	Cd	Cu	Ni	Pb	Zn
No		69	63	68	67	68	69
MIN		4.9	0.0	29.1	10.9	70.4	2.8
MAX		2888.7	4.8	484.5	57.5	1770.4	1303.3
Arth. Mean		676.5	1.8	140.5	33.3	424.6	164.2
Geo. Mean		401.5	1.5	116.6	31.5	301.1	125.1
Median		424.6	1.6	114.3	34.1	256.0	128.6
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	2	n/a	50	450*	n/a
No > ICRCL		68	7	29	0	20	3
No > CLEA		68	53	n/a	4	No	n/a
% > ICRCL		99%	11%	43%	0%	29%	4%
% > CLEA		99%	84%	n/a	6%	No	n/a
Mean > SGV		Yes	No	n/a	No	No*	n/a
US95 < SGV		FAIL	PASS	n/a	PASS	PASS*	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
MER 3	22	5.80	7.58	6.97	7.24

Organic Matter
LOI %
nt

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
MER 3	19.4	38.3	42.3	Clay Loam

Total Nitrogen
%N
0.221

As, Cu and to some extent Zn show a similar hotspot geometry, indicating elements are present in the same areas. Pb shows two well defined hotspots, but their location is independent to the elements mentioned above. Cd, although elevated, shows a diffuse pattern.

Figure 4.8.1. Contamination maps for MER 3

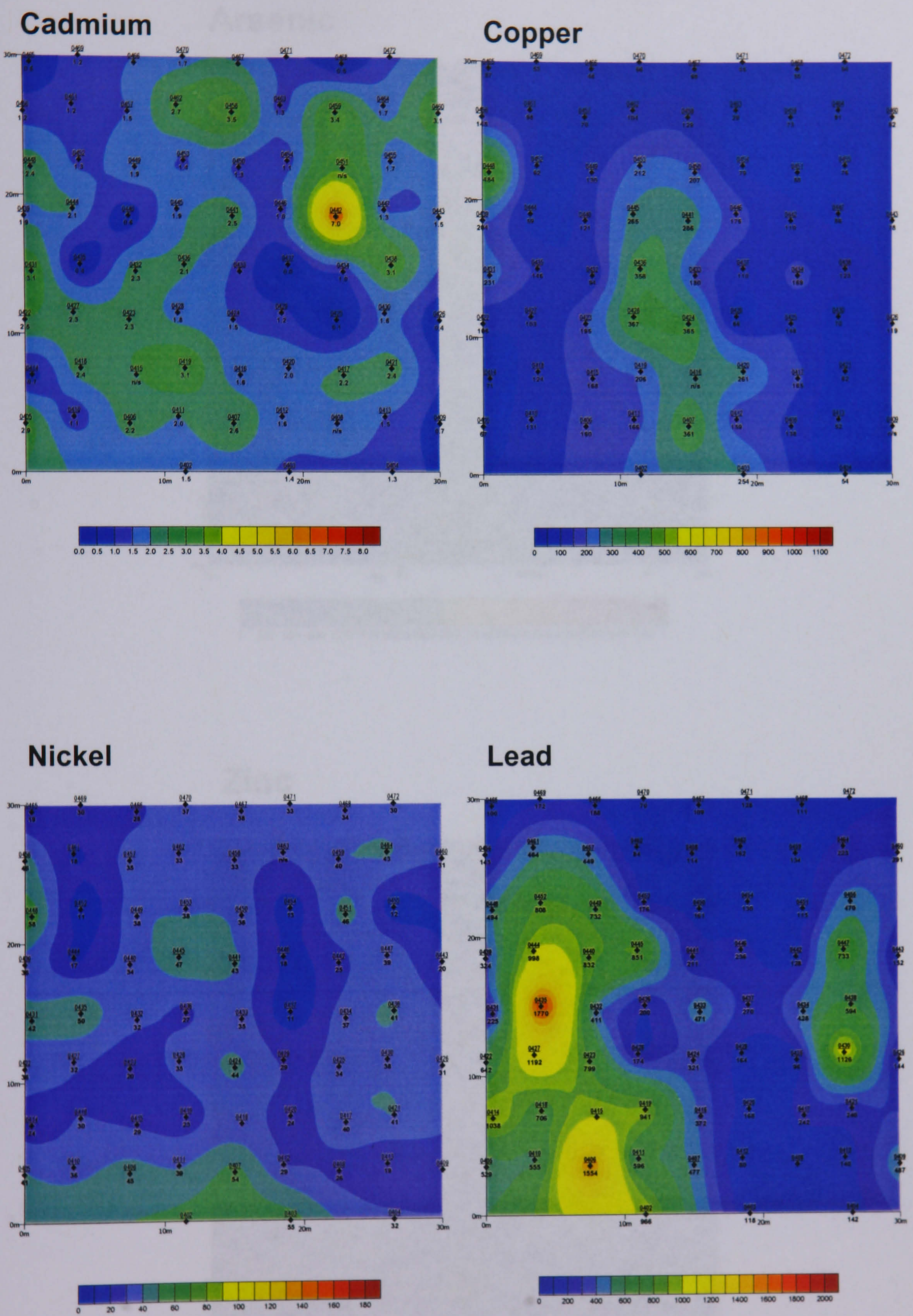
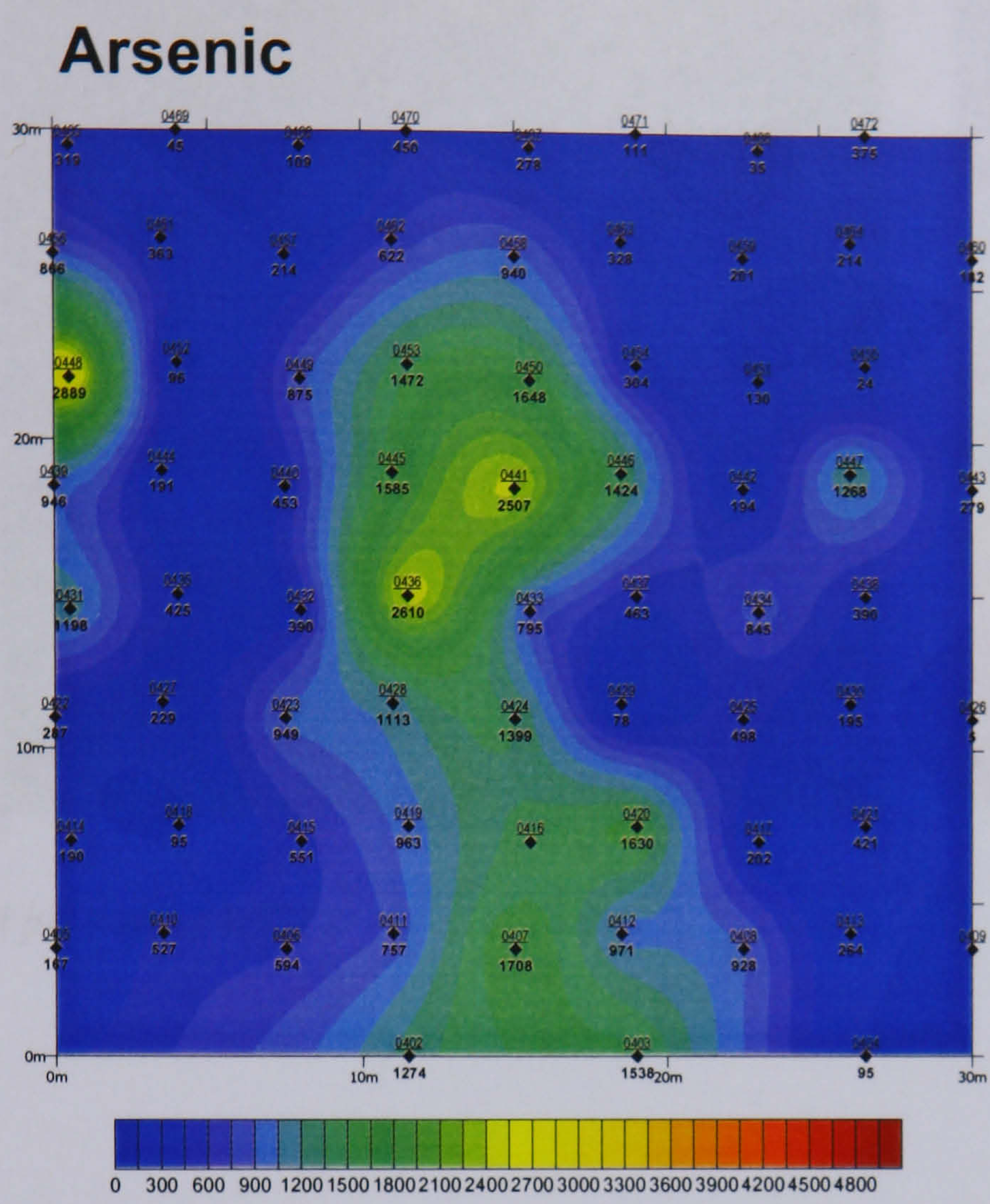
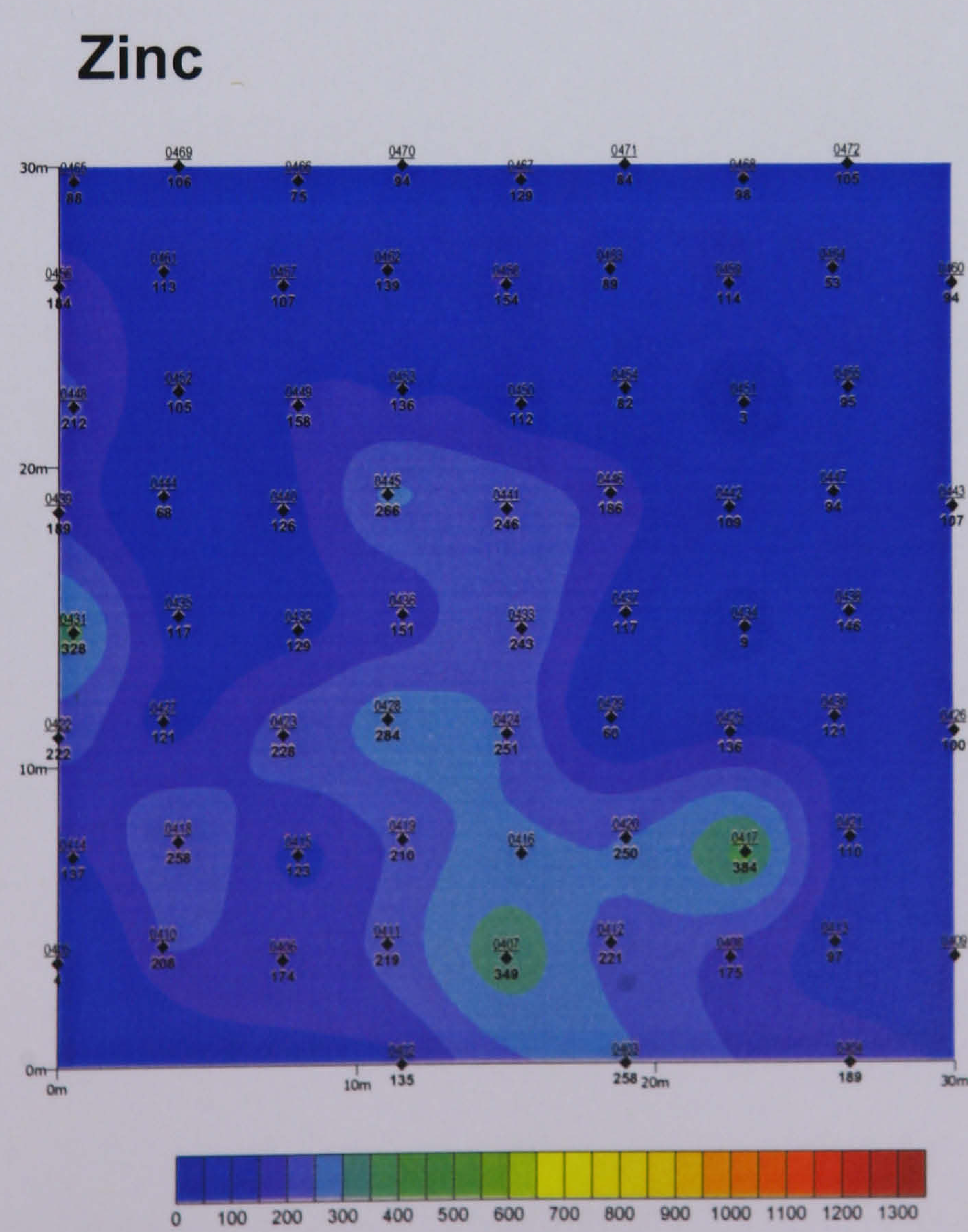


Figure 4.8.1. Contamination maps for MER 3 (cont'd)





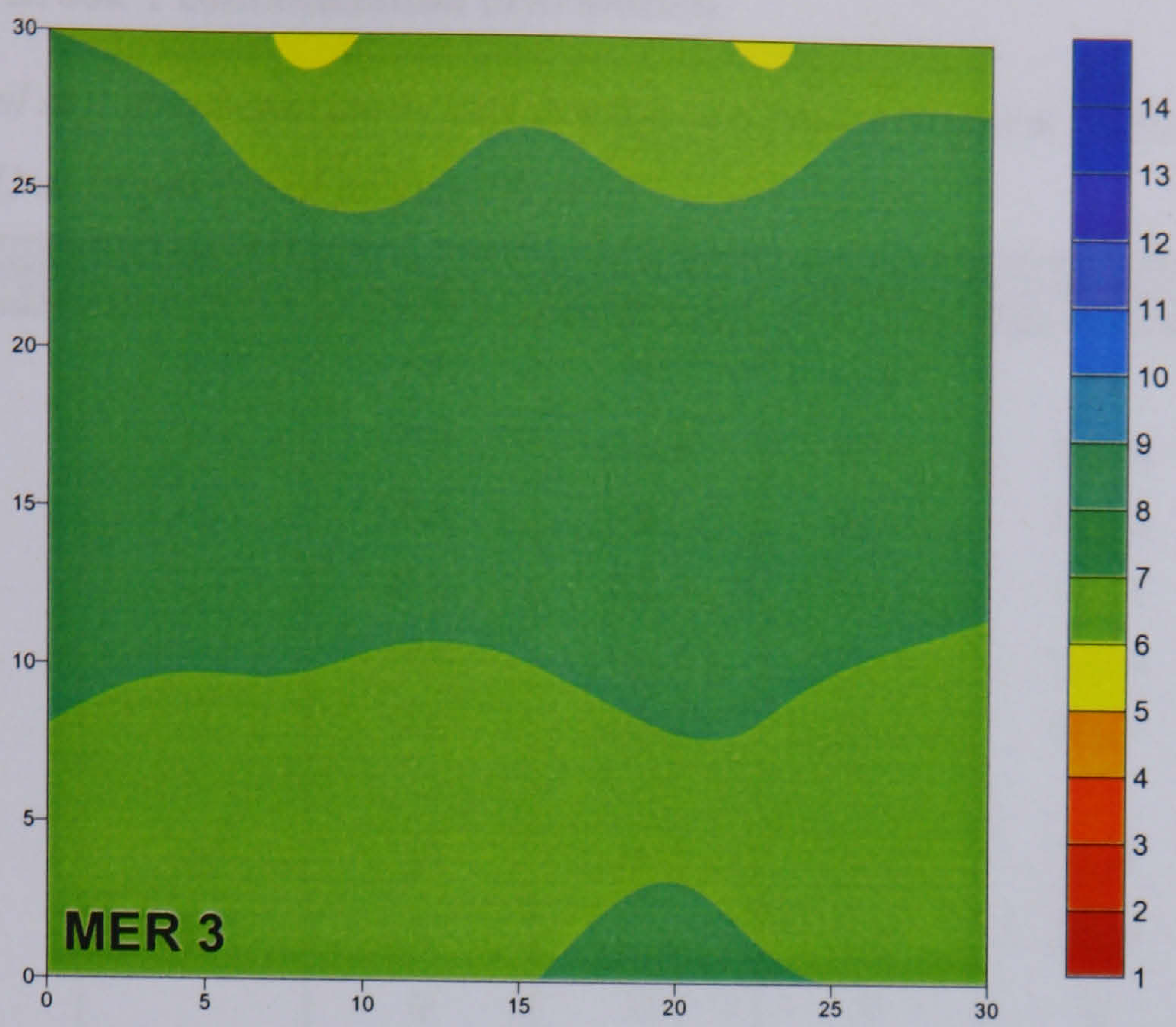


Figure 4.8.2. Soil pH map of MER 3

4.4.8 Sugar Brook 1 contamination distribution

Table 4.9. Total soil metal concentrations (mg/kg) and physiochemical parameters at Sugar Brook 1 (SUG1) at beginning of project (Year 1)

SUG 1		As	Cd	Cu	Ni	Pb	Zn
Number			14	14	14	14	14
MIN			<ld	38.6	19.1	108.8	127.5
MAX			1.5	75.7	38.4	484.8	372.8
Arth. Mean			0.3	52.9	30.3	174.1	189.2
Geo. Mean			N/a	52.0	29.8	160.0	181.0
Median			0.4	53.3	32.0	149.7	184.2
ICRCL		10[2]	3[2]	130[1]	70[1]	500[2]	300[1]
CLEA		20	1	n/a	50	450*	n/a
No > ICRCL			0	0	0	0	1
No > CLEA			3	n/a	0	No	n/a
% > ICRCL			0%	0%	0%	0%	7%
% > CLEA			21%	n/a	0%	No	n/a
Mean > SGV			No	n/a	No	No*	n/a
US95 < SGV			PASS	n/a	PASS	PASS*	n/a

[[1] ICRCL phytotoxic limits; [2] ICRCL trigger concentrations for domestic gardens / allotments]
Mean > SGV: Mean soil concentration higher than CLEA SGV for the element.
US95 < SGV: Is upper 95th percentile of mean soil concentration below (pass) or above (Fail) SGV.
* Based on geometric mean. Comparisons use geometric mean not arithmetic. (see Section 4.6 for full explanation of these terms).

	pH (H_2O)				
Site	n	MIN	MAX	Mean	Bulk Samp
SUG 1		nt	nt	nt	5.38

Organic Matter
LOI %
nt

	Soil Particle Size Distribution			
Site	% Clay	% Silt	% Sand	Soil Type
SUG 1	10.3	13.9	75.7	Silt loam

Total Nitrogen
%N
0.151

SUG 1 shows no significant contamination from any element. Where Cd has exceeded guidance on 3 occasions is more likely result of detection limitations and mean concentrations are very low.

Figure 4.9.1. Contamination maps for SUG 1.

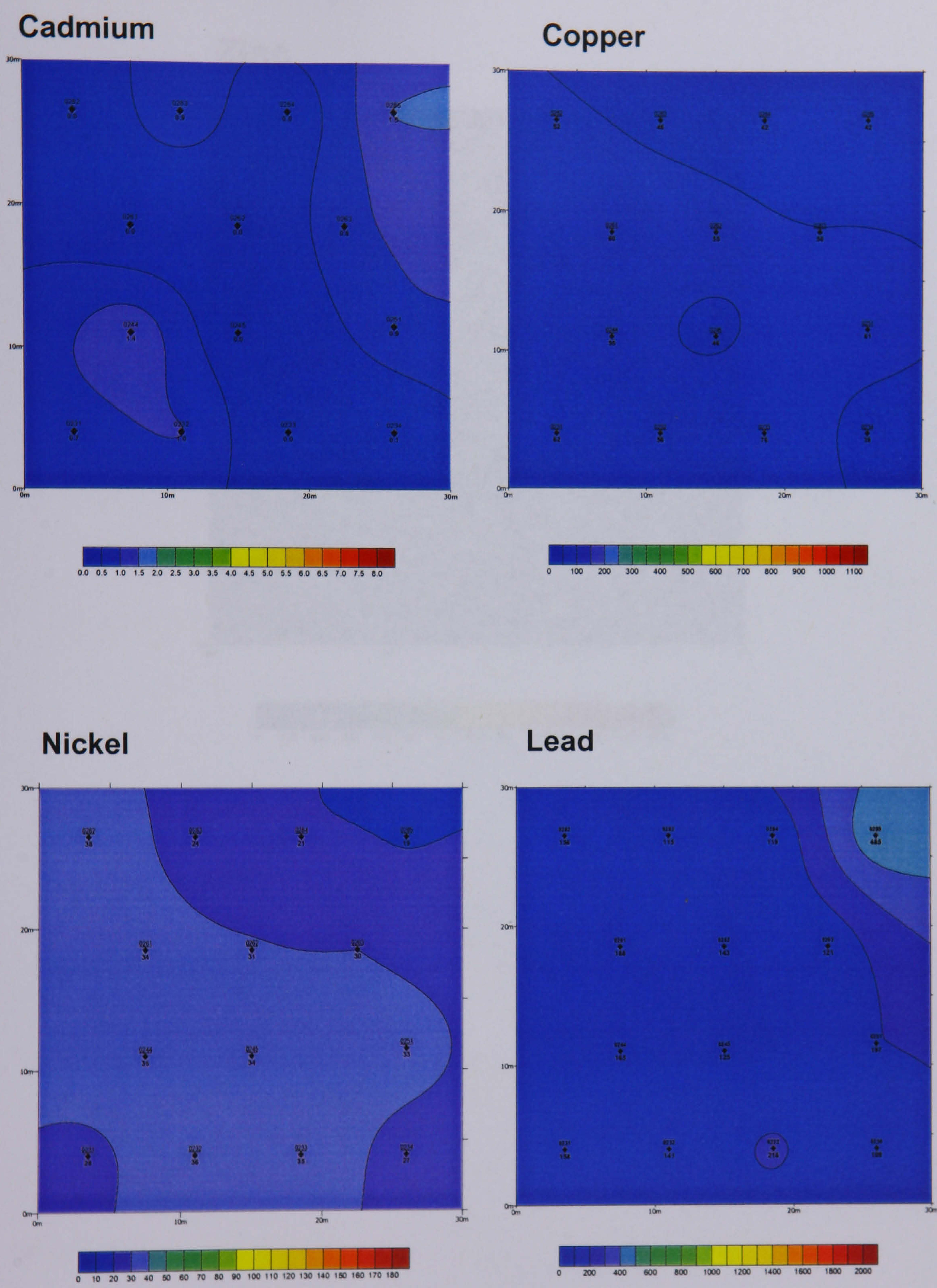


Figure 4.9.1. Contamination maps for SUG 1 (cont'd)

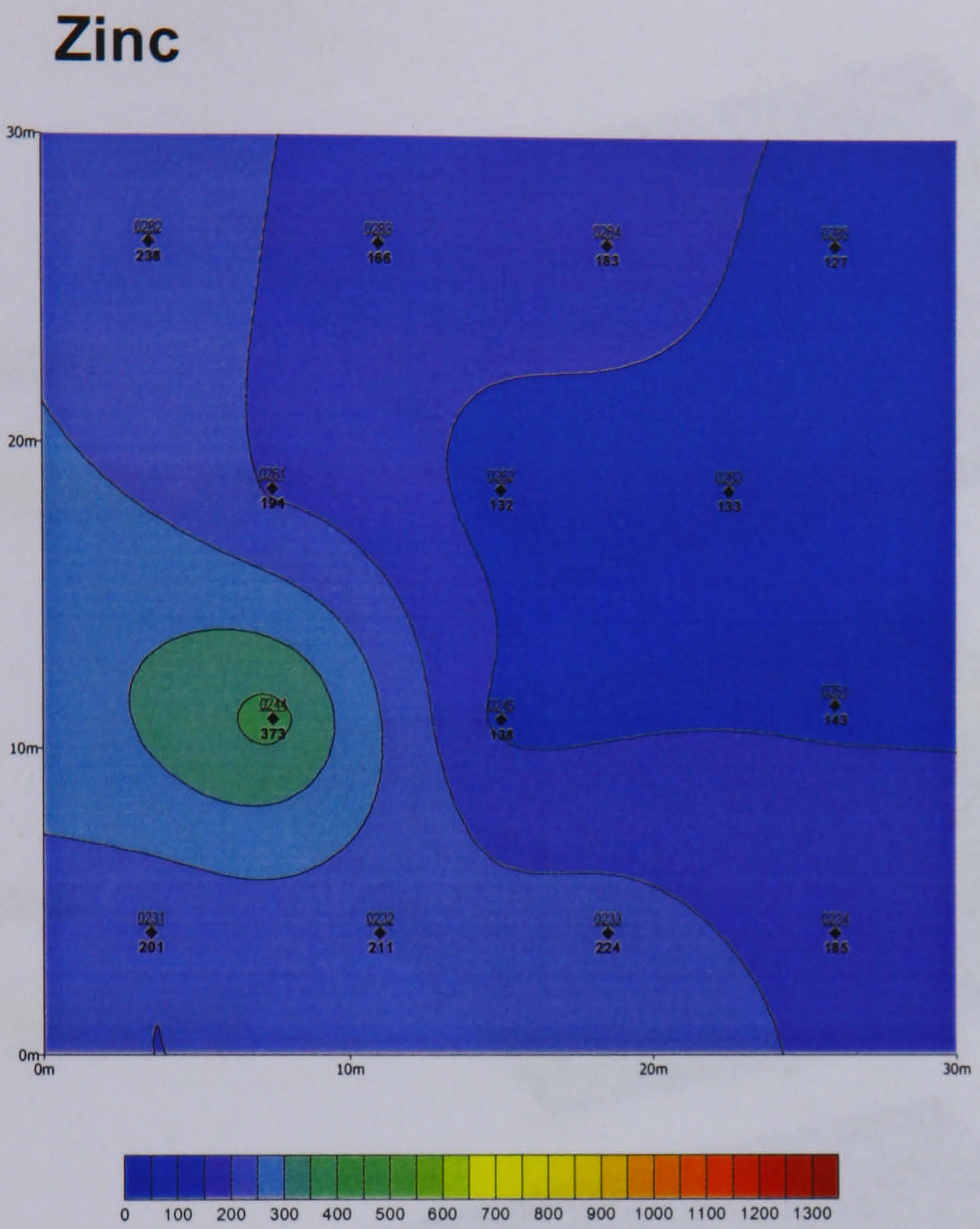
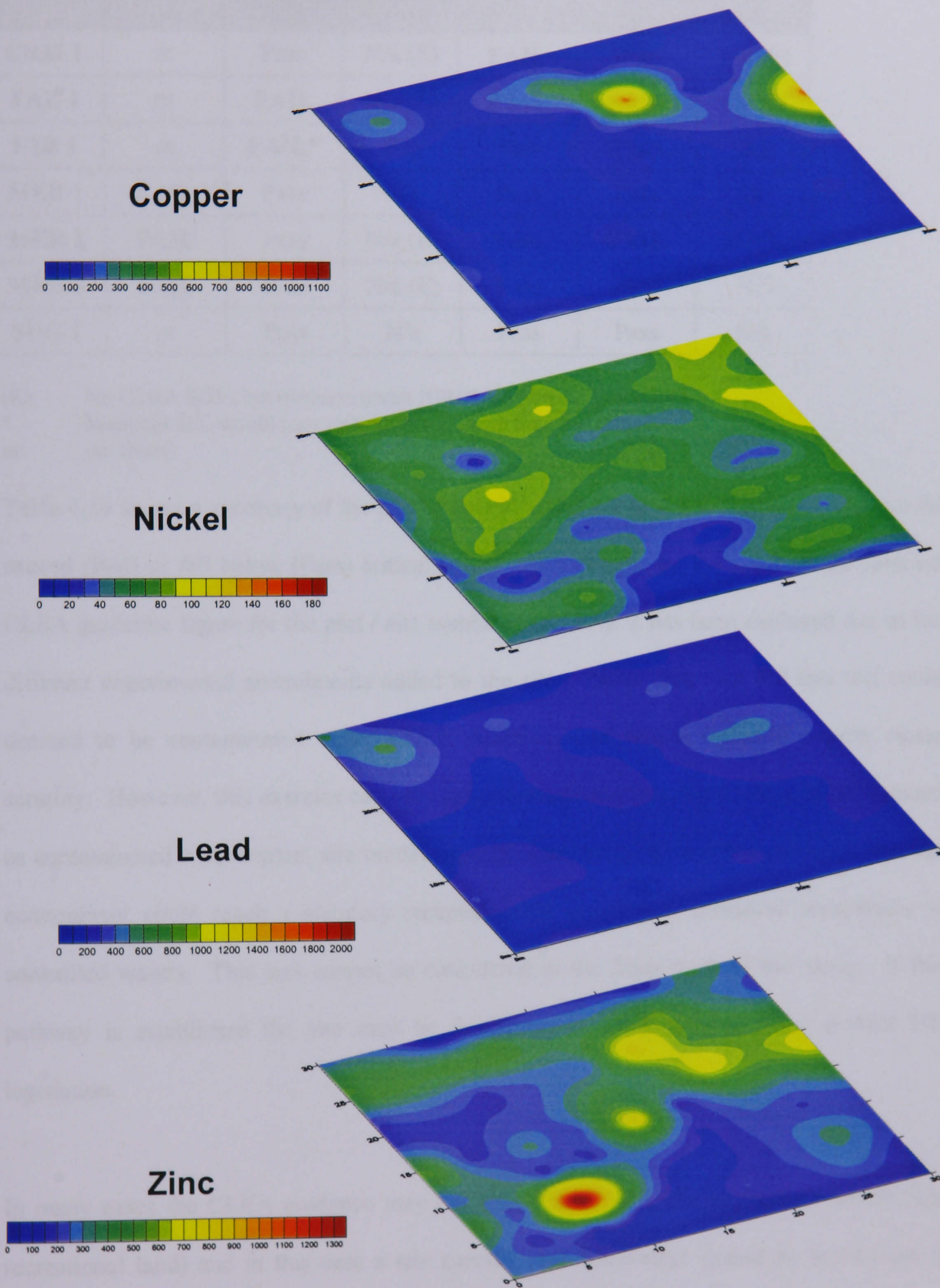


Figure 4.10.1. 3D stacking of maps used for visual contamination comparison and hotspot definition. Example shown is CRM1



4.5 Site contamination summary

Table 4.10. Summary of experimental plots that pass or fail under CLEA guidance.

	As	Cd	Cu	Ni	Pb	Zn
CRM 1	nt	Pass	N/a (E)	**FAIL**	Pass	N/a (E)
FAZ 1	nt	**FAIL**	N/a (E)	**FAIL**	**FAIL**	N/a (E)
KIR 1	nt	**FAIL***	N/a	Pass	Pass	N/a
MER 1	**FAIL**	Pass	N/a	Pass	Pass	N/a
MER 2	**FAIL**	Pass	N/a (E)	Pass	**FAIL**	N/a (E)
MER 3	**FAIL**	Pass	N/a (E)	Pass	Pass	N/a
SUG 1	nt	Pass	N/a	Pass	Pass	N/a

(E) No CLEA SGV, but elevated under ICRCL phytotoxic guidelines
* Marginal fail, would pass under more realistic land use criteria
nt not tested

Table 4.10 shows a summary of the data shown in tables 4.2 to 4.9 indicating if plots would exceed (Fail) or fall below (Pass) critical values when comparing the US95 to the selected CLEA guideline figure for the plot / site combination. FAZ 2 has been excluded due to the different experimental amendments added to the site. Those sites that fail this test could deemed to be contaminated under CLEA guidance, and would certainly require closer scrutiny. However, this exercise can only act as a screening tool, for a site to be designated as contaminated a conceptual site model must be constructed to identify pathways that the contaminant could reach a statutory receptor, such as humans, protected ecosystems or controlled waters. This task cannot be considered in the framework of this study. If this pathway is established the site may be designated as contaminated under current UK legislation.

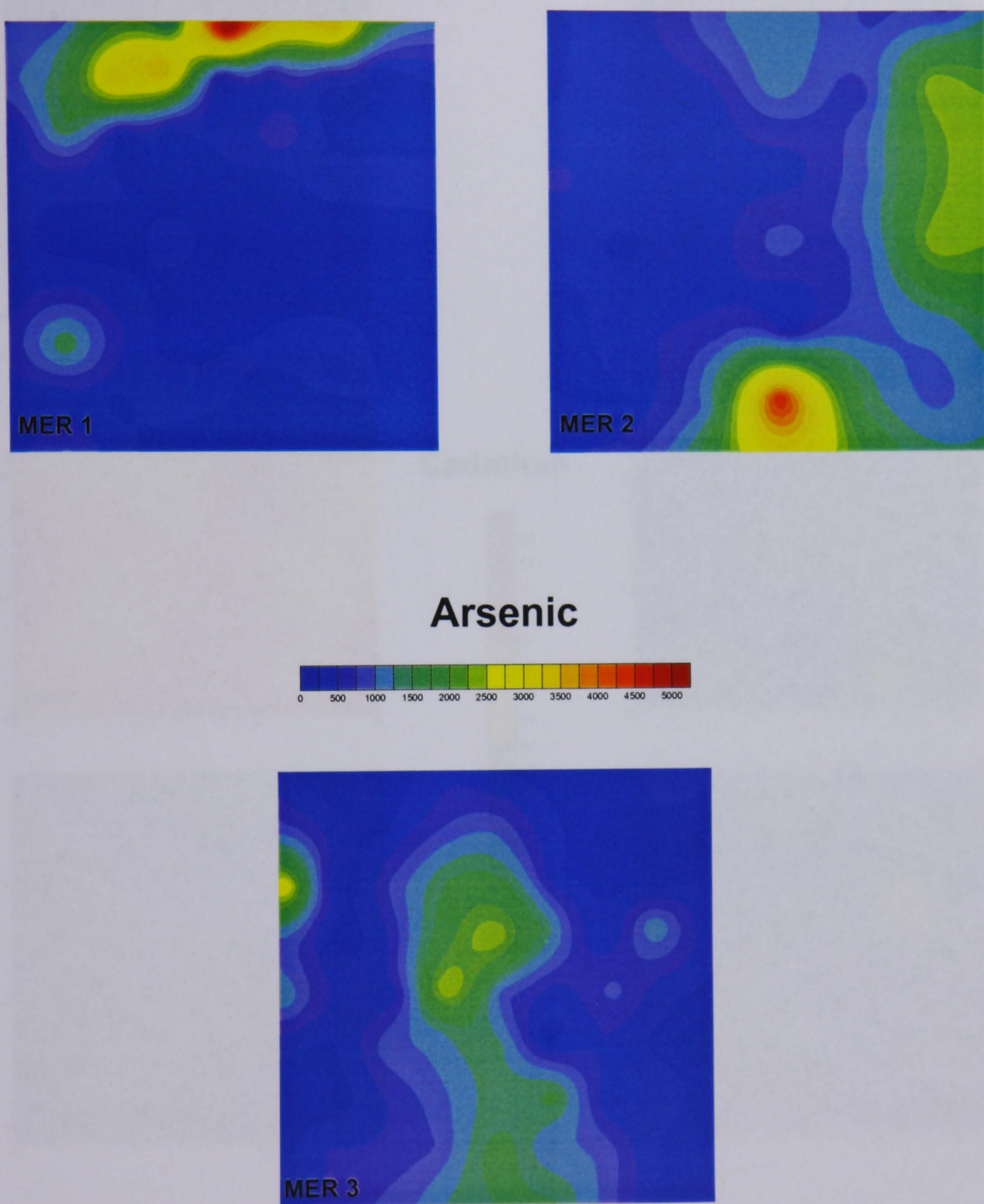
In many cases the CLEA guidance may not allow for a particular exposure scenario (e.g. recreational land) and in this case a site specific risk assessment should be carried out to ascertain the actual risk to those using the site, or other receptors such as ecosystems and watercourses. This step would be of critical importance if allowing public access to the land or developing the land in any way where exposure to soil contaminants would be an issue.

4.6 Contaminant mapping by element

This section presents the overall distribution of each element on all experimental plots.

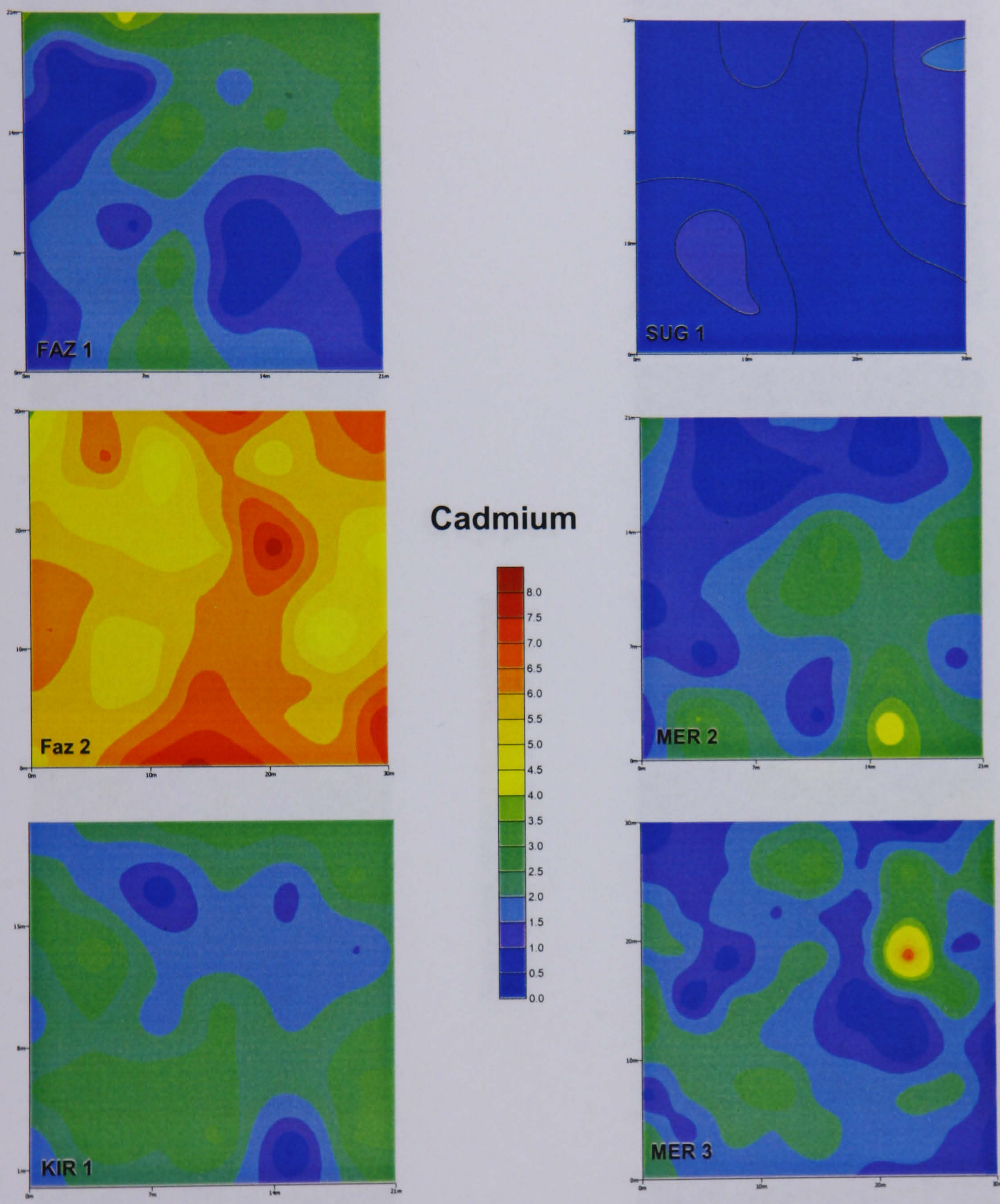
Arsenic

Figure 4.11. Contamination maps for arsenic (As) at different experimental plots



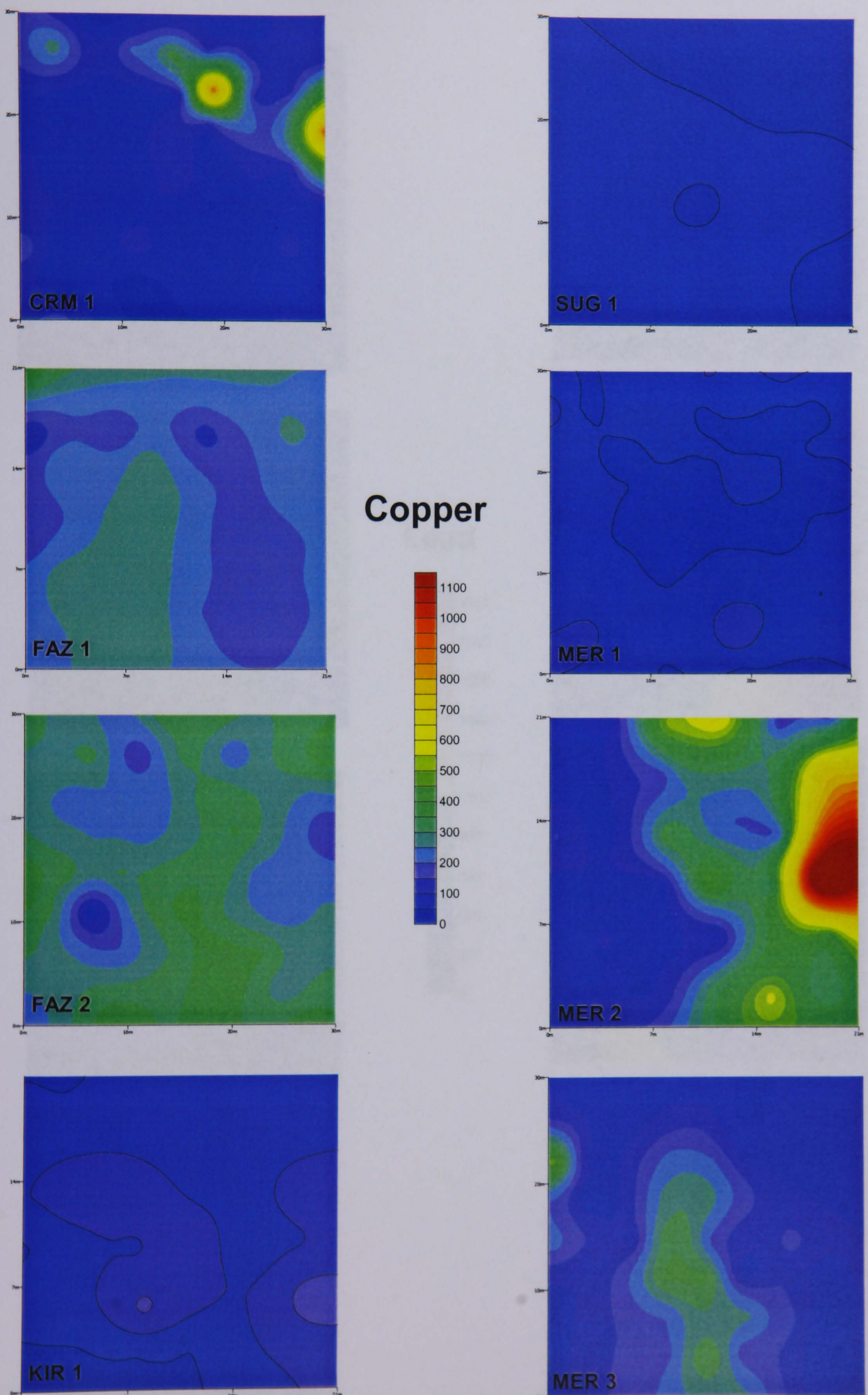
Cadmium

Figure 4.12. Contamination maps for cadmium (Cd) at different experimental plots



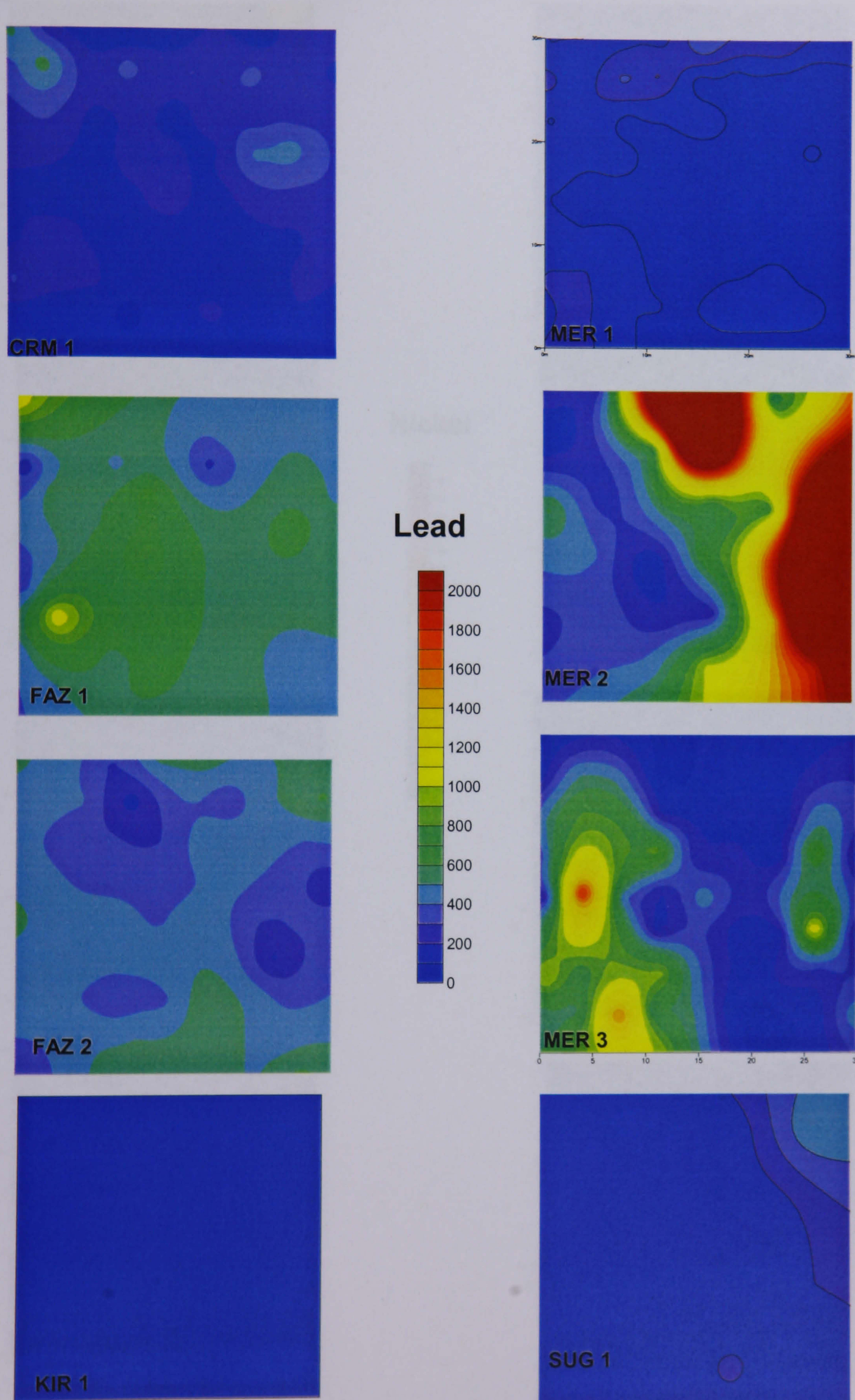
Copper

Figure 4.13. Contamination maps for copper (Cu) at different experimental plots.



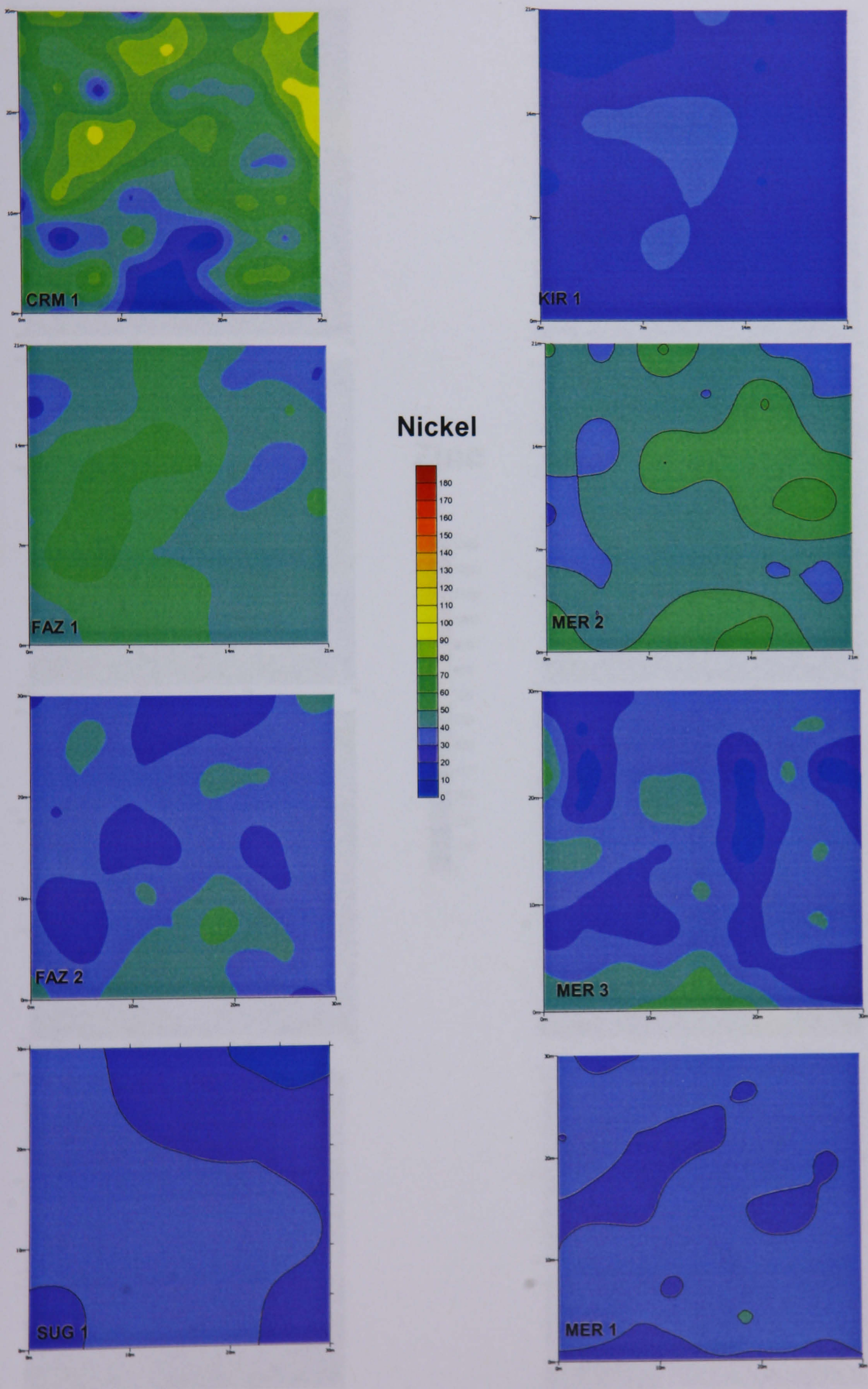
Lead

Figure 4.14. Contamination maps for lead (Pb) at different experimental plots.



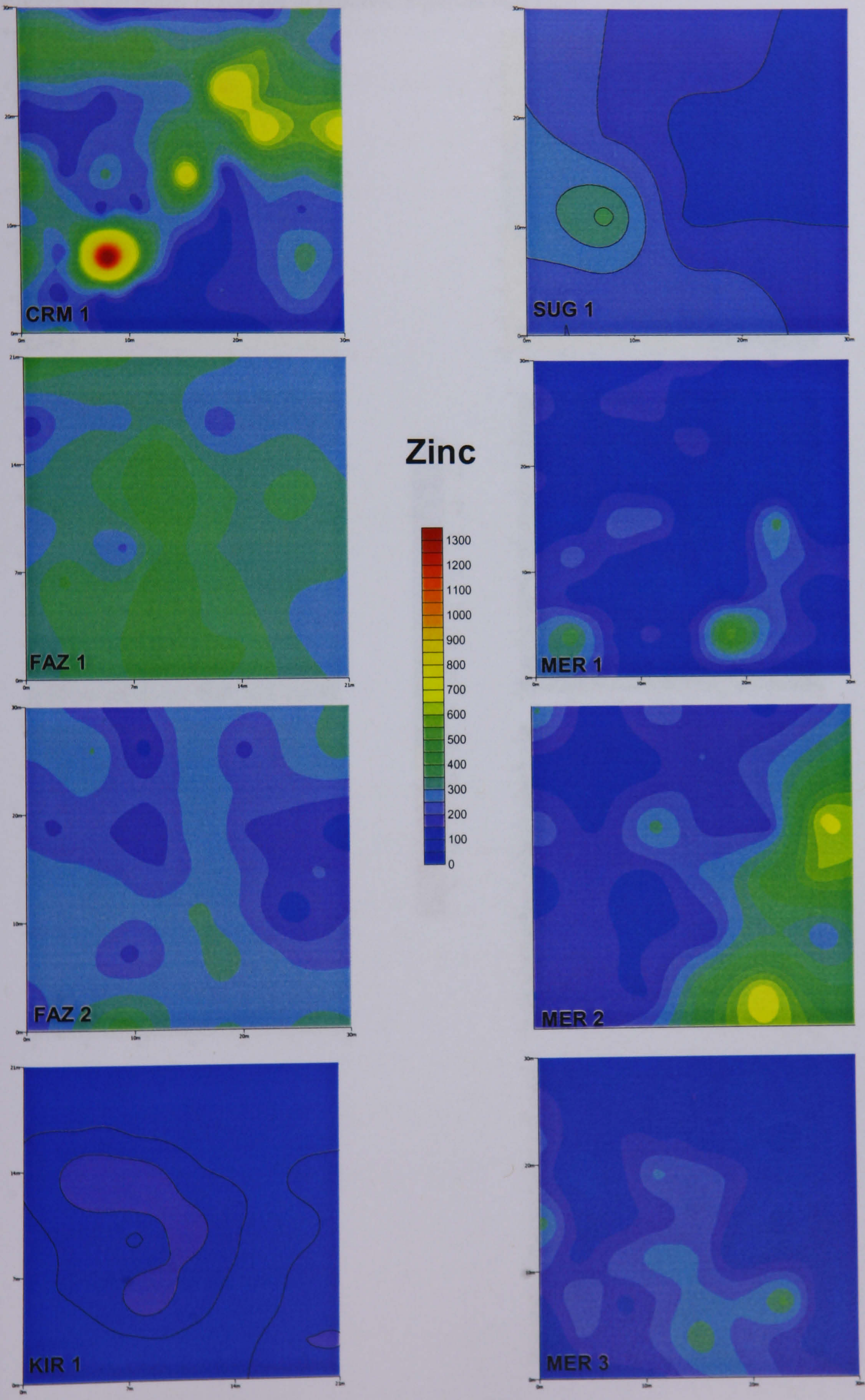
Nickel

Figure 4.15. Contamination maps for nickel (Ni) at different experimental plots



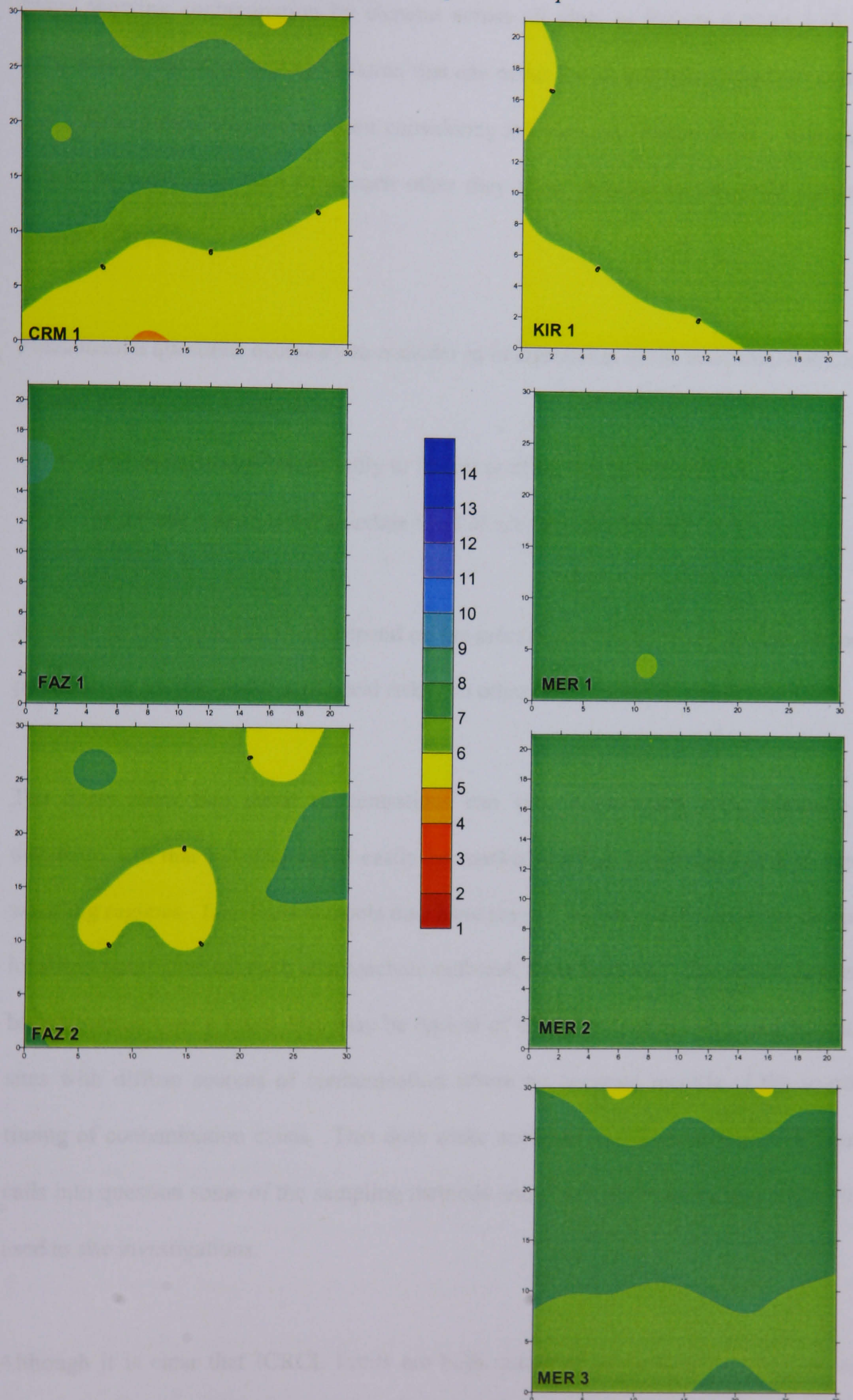
Zinc

Figure 4.16. Contamination maps for zinc (Zn) at different experimental plots



Soil pH

Figure 4.17 - Maps of soil pH at different experimental plots



4.7 Interpretation of maps

Maps showing contamination by element across all plots in figures 4.10 to 4.16 give an indication of the large spatial variation that can occur for an individual element on the same plot. This is most prominent when considering the plots on Merton Bank. Although each plot is no more than 80m from each other they show extreme variation for almost every element mapped.

Fundamental questions necessary to consider in interpretation of the contamination maps and onward investigations include:

- Are contaminant levels likely to inhibit or affect the growth of trees
- Is the site safe to allow a certain level of access to the public?

Answers to these questions will depend on the present and future use of the site, its location, proximity to people, wider ecological risks and other environmental issues.

The maps show that metal concentrations can vary dramatically over relatively small distances, and that hotspots could easily be masked through composited or less extensive sampling regimes. Localised hotspots may have several origins, including waste dumping or localised point sources, such as a leachate outbreak from landfills. The results suggest that high variation over a small area may be typical of urban brownfield sites and indicative of sites with diffuse sources of contamination where no accurate records of the source and timing of contamination exists. This does make accurate site characterisation difficult and calls into question some of the sampling methods and standard protocols that are commonly used in site investigations.

Although it is clear that ICRCL limits are both outdated and inaccurate when relating to phytotoxicity of trees, they still provide one of the few existing benchmarks of contamination. At the very least, they provide a pragmatic guide and may demonstrate a significant deviation from background soil concentrations of metals. Their replacement with CLEA takes no account of either trees or, more specifically public access amenity spaces, leaving a void in the planning and regulatory system.

4.8 Conclusions and discussion

The sampling and mapping techniques used have been successful in allowing a focused approach for future monitoring. Identifying a range elevated metal hotspots and species growing on them will allow more focused and relevant monitoring in a large scale field experiment.

The mapping study has demonstrated the highly variable nature of brownfield soils over a small spatial distances. The spatial variability probably reflects historical dumping and reclamation practices and the results reinforce the importance of correct sampling procedures. Sampling at lower densities such as 20m intervals as suggested in some guidance may allow hotspots to be missed altogether, providing additional risk for the environment and people using the site.

Issues concerning sampling type, locations, numbers, nature of hazard and possible risk are all considerations for any sampling exercise on contaminated land, and will frequently be driven by cost and end use of the land (Andronikov *et al.* 2000; Bacon and Hudson 2001; Nortcliff 2001)

The data outline potential flaws in the current regulatory system of defining contaminated land in the context of community orientated projects. Although a human health, risk based, model is welcome compared to previous guidance, the lack of a range of land use types is likely to cause problems. At its most simplistic this will either under or overcompensate by forcing an inappropriate land use, for example in the present study guidance figures are considered in a very robust way, by adopting the most sensitive land use. It is possible that some sites, by virtue of their remoteness or limited access (e.g. Kirby Moss), could be assessed under less stringent measures. At this site it is possible to justify SGV's for Cadmium of both 1ppm (residential, with uptake at pH 6.5) and 30ppm (residential, no uptake), adoption of the latter results in a 'clean' site, whereas using the former causes 94% of the samples to exceed the SGV. At other sites (e.g. MER and CRM) it may be justified to assess in comparison to acceptable 'garden' levels, due to their frequent and continued use by the community. Site-specific conceptual models may have to be derived for sites where public access will be at this level. Community forestry focuses on public access to areas planted with trees or other developing ecosystems. A compromise may be to fence certain 'hotspot' areas of the site, if assessment is carried out on a suitable scale, to prevent public access. Trees could also be planted over hotspots, dense, un-thinned planting as found in coppice systems may reduce public access to certain areas, effectively acting as a barrier between humans and the soil, breaking the source, pathway, receptor model.

It would be possible to carry out site specific assessments using the algorithms and probabilistic models provided with the CLEA, CLR10 report (DEFRA and Environment Agency 2002e) or another risk based framework. However, it is uncertain if data exists on accurate community exposure by use of these sites, or the role of trees and ecosystems that may or may not be accumulating metals on the site.

Chapter 5: Modelling and Interactions

Introduction

The identification and selection of willows and poplars for phytoremediation has been largely based on screening potentially suitable taxa in either glasshouse or small-scale field-experiments. Field studies have been limited to a single or very few sites with specific contamination issues, such as sewage sludge application, fertiliser residue, mine spoil or other relatively easily defined contaminated soil systems. The applicability of these studies to brownfield sites is questionable. Of equal interest is whether species or taxa share growth and uptake characteristics across different sites, and if predictive measurements of wider usage are possible.

This chapter aims to assess the range of field data collected over three years of experimental monitoring using a range of statistical and modelling techniques.

Plate 5.1. Monitoring tree growth at the end of Year 1 for future modeling work

Data Collection and Analysis

Data collected from nine field plots at six independent sites over a 3-year period have been used to examine trends of metal interactions with trees. Detailed information on data collected and analytical methodology is provided in Chapter 3, but a summary of the variables examined in this chapter, their sampling frequency and sampling unit is provided in Table 5.1 below. Where no entry is made, that particular variable was not sampled at that time period.

Table 5.1. Summary of data collection frequencies used in field monitoring of experimental plots for 3 years and variables used for analysis in this chapter.

Variable	Description	Year 1	Year 2	Year 3	Unit
Total Soil Trace Metals	HNO_3 digestion for total soil metals	Before planting			Whole plot
EDTA soil extractable Trace Metals	EDTA extractable soil metals	Before planting		End of year 3	Hotspot areas
$CaCl_2$ soil extractable Trace Metals	CaCl2 soil extractable metals	Before planting			Hotspot areas
Foliar Trace Metals	Foliage metal concentration and content		End of year 2	End of year 3	Selected trees
Stem Trace Metals	Stem metal concentration and content			End of year 3 (post harvest)	Selected trees
Biomass	ODT ha^{-1} Biomass			End of year 3	Whole plot
Mortality	% Mortality	End of year 1	End of year 2	End of year 3	Whole plot
pH	Soil pH	Before planting			Whole plot

5.1 Soil Hotspot Areas

Areas for additional soil analysis were formed from hotspots of contaminant types as defined in Chapter 4. In order to create contamination maps each soil sample was analysed for total soil metal concentration using a HNO_3 digest. A subset of these samples within elevated metal concentration hotspots, were also analysed for $CaCl_2$ and EDTA soil extractable metals via a 2-stage process described in Chapter 3.

The data analysed here are used to assess:

(i) If variability in soil metal concentration is significant when examining hotspot areas rather than whole plots

(ii) The relationship between different extraction procedures on brownfield soils, and

(iii) Their usefulness in monitoring and modeling phytoremediation process.

Results

Tables 5.1.1 to 5.1.8 show analysis of sub samples taken within hotspot zones prior to tree planting in Year 1. All results are in mg kg^{-1} unless indicated. EDTA1 refers to EDTA concentrations taken from Year 1 as these measurements were taken over several time periods and will be compared later in the chapter. Percentages of $CaCl_2$ and EDTA are shown as a function of total metal extraction using HNO_3 digest and in the case of $CaCl_2$ also as a function of EDTA extraction. These data are summarised in Figures 5.1 and 5.2

Table 5.1.1. Merton Bank 1 (MER 1) Hotspot soil analysis in Year 1 prior to tree planting. All figures in mg kg^{-1} unless otherwise indicated

MER 1	Arsenic			Cadmium			Copper		
	Total	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**
n	15	15	15		15.0	15.0	15	15	15
MIN	32.2	0.1	2.4		0.0	0.1	38.2	0.1	7.9
MAX	5265.6	31.8	173.6		0.0	1.4	144.8	0.3	31.0
Mean	1206.7	5.5	43.2		0.0	0.5	89.5	0.2	15.3
Median	371.6	1.0	16.6		0.0	0.3	90.9	0.2	14.0
Std Dev	1622.5	9.2	54.1		0.0	0.5	39.6	0.1	7.2
% of Total		**0.5**	**3.6**					**0.2**	**17.1**
% of EDTA		**12.8**			**0.5**			**1.2**	**-**

	Lead			Nickel			Zinc		
	Total	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**
n	15	15	15	15	15	15	15	15	15
MIN	42.5	0.0	11.8	22.3	0.2	0.3	90.1	0.9	5.2
MAX	394.7	0.2	72.3	41.4	0.5	1.3	339.2	1.3	86.6
Mean	183.1	0.1	39.3	34.1	0.3	0.8	214.8	1.1	31.8
Median	201.5	0.0	41.9	35.4	0.3	0.7	231.9	1.1	15.6
Std Dev	104.0	0.1	22.0	6.1	0.1	0.3	77.7	0.2	32.7
% of Total		**0.0**	**21.5**		**0.8**	**2.3**		**0.5**	**14.8**
% of EDTA		**0.2**	**-**		**36.3**	**-**		**3.4**	**-**

Table 5.1.2. Merton Bank 2 (MER 2) Hotspot soil analysis in Year 1 prior to tree planting. All figures in mg kg^{-1} unless otherwise indicated

MER 2	Arsenic			Cadmium			Copper		
	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1
n	13	13	13	16	13	13	13	13	13
MIN	230.0	0.1	3.5	0.8	0.0	0.2	98.4	0.0	26.6
MAX	4611.9	3.7	114.9	4.5	0.0330	0.5	1146.5	0.9	214.1
Mean	1527.3	1.6	38.7	2.0	0.004	0.3	541.1	0.4	101.2
Median	1200.3	1.0	36.6	1.8	0.001	0.3	364.7	0.5	95.5
Std Dev	1184.1	1.2	33.7	1.1	0.0	0.1	343.8	0.3	59.7
% of Total		**0.1**	**2.5**		**0.2**	**15.0**		**0.1**	**18.7**
% of EDTA		**4.1**			**1.2**			**0.4**	

	Nickel			Lead			Zinc		
	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1
n	13	13	13	13	13	13	13	13	13
MIN	38.3	0.1	0.4	382.1	0.0	85.4	169.5	0.7	15.3
MAX	62.8	0.3	2.7	3641.5	0.3	1576.6	729.8	1.6	86.7
Mean	49.5	0.2	1.5	2087.8	0.1	645.6	389.1	1.0	40.2
Median	50.3	0.2	1.6	1914.5	0.1	572.8	331.9	0.9	33.2
Std Dev	9.2	0.1	0.7	1276.2	0.1	464.9	199.8	0.2	22.4
% of Total		**0.4**	**3.0**		**0.0**	**30.9**		**0.2**	**10.3**
% of EDTA		**14.3**			**0.0**			**2.4**	

Table 5.1.3. Merton Bank 3 (MER 3) Hotspot soil analysis in Year 1 prior to tree planting. All figures in mg kg^{-1} unless otherwise indicated.

MER 3	Arsenic			Cadmium			Copper		
	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1
n	19	19	19	19	19	19	18	19	19
MIN	189.9	0.0	3.1	0.4	0.0	0.1	59.4	0.0	12.1
MAX	2888.7	5.6	67.8	2.6	0.0	0.3	484.5	0.3	56.8
Mean	1423.5	2.1	24.3	1.9	0.0	0.2	259.4	0.2	34.0
Median	1538.4	2.1	20.4	1.9	0.0	0.2	263.2	0.2	35.9
Std Dev	873.4	1.6	16.3	0.6	0.0	0.1	129.1	0.1	11.6
% of Total		**0.1**	**1.7**		**0.0**	**11.8**		**0.1**	**13.1**
% of EDTA		**8.6**			**0.3**			**0.5**	
	Nickel			Lead			Zinc		
	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1
n	18	19	19	19	19	19	19	19	19
MIN	16.8	0.1	0.7	118.1	0.0	43.0	68.3	0.5	7.2
MAX	57.5	0.2	2.3	1770.4	0.2	265.3	1303.3	0.8	29.3
Mean	41.7	0.2	1.3	656.2	0.1	174.7	306.8	0.7	19.7
Median	44.2	0.2	1.1	493.8	0.1	190.0	212.1	0.8	22.4
Std Dev	12.7	0.0	0.6	490.3	0.1	68.0	356.5	0.1	6.4
% of Total		**0.4**	**3.1**		**0.0**	**26.6**		**0.2**	**6.4**
% of EDTA		**14.4**			**0.0**			**3.8**	

Table 5.1.4. Cromdale Grove 1 (CRM1) Hotspot soil analysis in Year 1 prior to tree planting. All figures in mg kg^{-1} unless otherwise indicated.

CRM 1	Copper			Nickel			Lead			Zinc		
	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1
n	17	17	17	17	17	17	17	17	17	17	17	17
MIN	10.4	0.1	17.6	44.3	0.2	1.2	111.2	0.0	36.5	95.1	1.3	16.6
MAX	883.5	1.3	531.9	109.4	2.6	4.7	560.1	0.2	198.9	826.6	16.2	254.7
Mean	287.4	0.5	120.2	76.1	1.0	2.2	290.8	0.1	108.6	496.4	4.8	80.4
Median	164.3	0.3	72.5	75.5	0.8	1.8	259.5	0.1	97.3	485.0	3.7	57.5
Std Dev	293.5	0.4	158.0	22.1	0.8	1.1	126.2	0.0	55.4	222.7	4.1	71.5
% of Total		**0.2**	**41.8**		**1.3**	**2.9**		**0.0**	**37.3**		**1.0**	**16.2**
% of EDTA		**0.4**			**44.7**			**0.1**			**5.9**	

Table 5.1.5. Fazakerley 1 (FAZ 1) Hotspot soil analysis in Year 1 prior to tree planting. All figures in mg kg^{-1} unless otherwise indicated.

FAZ 1	Cadmium			Copper			Nickel		
	Total	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**
n	15	15	15	15	15	15	15	15	15
MIN	0.8	0.0	0.7	210.7	0.4	91.2	42.2	0.2	1.9
MAX	4.2	0.2	1.3	404.7	1.1	177.2	66.9	1.4	3.9
Mean	2.6	0.0	0.9	269.6	0.8	123.5	54.1	0.4	2.9
Median	2.4	0.0	0.9	267.1	0.7	120.4	52.6	0.3	3.1
Std Dev	0.9	0.0	0.2	46.9	0.2	25.3	7.4	0.3	0.6
% of Total		**1.2**	**35.6**		**0.3**	**45.8**		**0.8**	**5.4**
% of EDTA		**3.3**			**0.6**			**14.5**	

	Lead			Zinc		
	Total	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**
n	15	15	15	15	15	15
MIN	493.5	0.0	183.7	281.0	0.9	38.2
MAX	1102.6	0.2	407.4	536.1	3.8	80.7
Mean	684.2	0.1	303.7	394.6	1.5	59.6
Median	627.5	0.1	304.3	389.7	1.4	60.0
Std Dev	181.6	0.0	66.2	72.2	0.7	14.9
% of Total		**0.0**	**44.4**		**0.4**	**15.1**
% of EDTA		**0.0**			**2.4**	

Table 5.1.6. Fazakerley 2 (FAZ 2) Hotspot soil analysis in Year 1 prior to tree planting. All figures in mg kg^{-1} unless otherwise indicated

FAZ 2	Cadmium			Copper			Nickel		
	Total	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**
n	16	14	14	16	14	14	16	14	14
MIN	3.7	0.1	1.4	51.5	0.7	84.6	26.7	0.6	2.0
MAX	7.9	0.4	3.1	408.9	2.2	210.6	178.5	2.4	3.1
Mean	6.4	0.2	2.3	322.8	1.1	151.1	47.6	1.3	2.5
Median	6.5	0.2	2.2	338.6	1.0	148.5	39.3	1.2	2.4
Std Dev	1.1	0.1	0.4	85.3	0.4	29.4	35.5	0.4	0.4
% of Total		**3.5**	**35.1**		**0.3**	**46.8**		**2.7**	**5.2**
% of EDTA		**10.1**			**0.7**			**52.7**	

	Lead			Zinc		
	Total	**$CaCl_2$**	**EDTA1**	**Total**	**$CaCl_2$**	**EDTA1**
n	16	14	14	16	14	14
MIN	344.8	0.0	125.2	187.7	1.6	28.1
MAX	612.6	0.2	261.0	463.1	6.8	78.0
Mean	490.8	0.1	201.3	291.2	3.5	45.8
Median	491.5	0.1	201.6	285.3	3.1	43.4
Std Dev	65.1	0.1	32.1	56.7	1.8	13.3
% of Total		**0.0**	**41.0**		**1.2**	**15.7**
% of EDTA		**0.0**			**7.7**	

Table 5.1.7. Kirby Moss 1 (KIR1) Hotspot soil analysis in Year 1 prior to tree planting. All figures in mg kg^{-1} unless otherwise indicated.

KIR 1	Cadmium			Copper			Nickel		
	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1
n	12	10	10	12	10	10	12	10	10
MIN	1.4	0.0	0.3	48.5	0.1	24.1	12.2	0.4	1.5
MAX	3.4	0.1	0.6	163.0	0.4	77.3	37.8	0.7	3.1
Mean	2.7	0.1	0.4	106.4	0.2	53.5	28.9	0.5	2.6
Median	3.0	0.1	0.4	107.1	0.2	56.3	30.2	0.4	2.8
Std Dev	0.7	0.0	0.1	38.8	0.1	15.7	7.5	0.1	0.5
% of Total		**1.9**	**14.7**		**0.2**	**50.3**		**1.6**	**9.1**
% of EDTA		**13.0**			**0.4**			**17.4**	

	Lead			Zinc		
	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1
n	11	10	10	12	10	10
MIN	18.4	0.1	14.6	65.3	2.1	13.6
MAX	80.5	0.2	34.1	199.3	4.8	75.2
Mean	52.3	0.1	22.7	129.1	3.6	31.2
Median	55.0	0.1	21.3	112.2	3.6	24.1
Std Dev	18.0	0.0	7.0	55.7	1.0	18.2
% of Total		**0.2**	**43.4**		**2.8**	**24.1**
% of EDTA		**0.6**			**11.5**	

Table 5.1.8. Corus 1 (COR 1) Hotspot soil analysis in Year 1 prior to tree planting. All figures in mg kg^{-1} unless otherwise indicated.

COR 1	Copper			Nickel			Lead			Zinc		
	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1	Total	$CaCl_2$	EDTA1
n	8	8	8	8.0	8	8	8	8	8		8	8
MIN	103.1	0.2	13.9	78.6	0.4	6.4	286.7	0.1	64.0	310.1	1.4	42.3
MAX	175.0	0.4	40.4	111.6	1.7	10.7	496.8	0.2	82.8	449.5	3.7	69.0
Mean	140.9	0.3	23.4	93.6	1.2	8.5	385.3	0.1	72.1	378.5	2.1	52.9
Median	143.3	0.3	19.5	95.5	1.2	8.3	375.6	0.1	71.0	396.2	1.8	51.5
Std Dev	23.7	0.1	9.2	11.1	0.376	1.731	66.8	0.0	5.6	49.26	0.741	10.21
% of Total		**0.2**	**16.6**		**1.2**	**9.1**		**0.0**	**18.7**		**0.6**	**14.0**
% of EDTA		**1.3**			**13.6**			**0.2**			**4.0**	

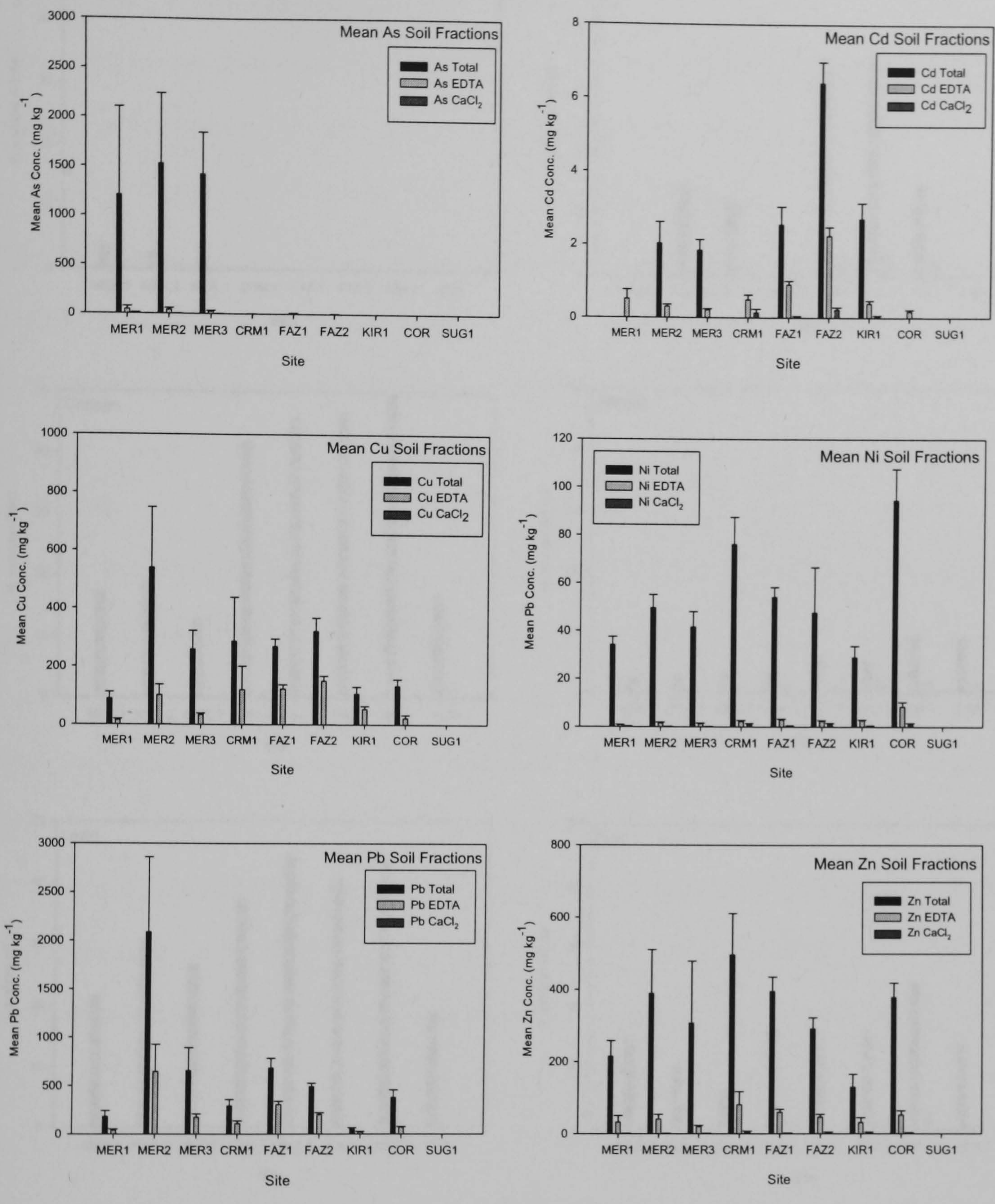


Figure 5.1.1. Extractable soil fractions of metals using HNO_3, EDTA and $CaCl_2$ extraction methods on hotspot areas in Year 1 prior to tree planting for each of the 9 plots. Error bars show 95% Confidence Intervals.

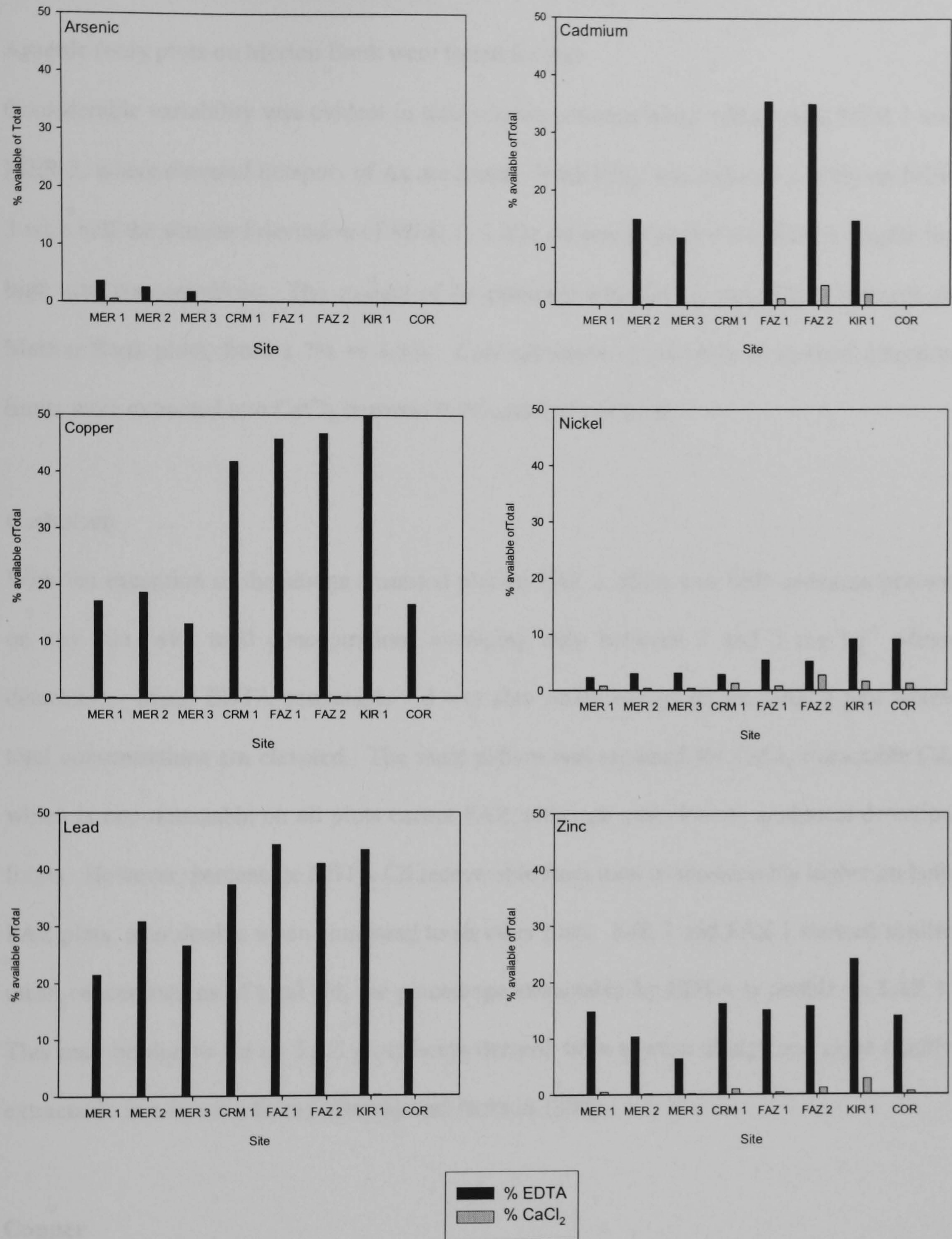


Figure 5.1.2. EDTA and $CaCl_2$ extractable metals as a percentage of total (HNO_3 extractable) soil metal concentrations in hotspot areas at Year 1 prior to tree planting

Arsenic (only plots on Merton Bank were tested for As)

Considerable variability was evident in total arsenic concentrations within plots MER 1 and MER 2, where elevated hotspots of As are found. Variability was reduced slightly on MER 3 with half the standard deviation of MER 1. Little As was extracted into EDTA despite the high total concentrations. The amount of As extracted with EDTA varied little between all Merton Bank plots, from 1.7% to 3.5%. Concentrations of As close to method detection limits were extracted into $CaCl_2$, between 0.5% and 0.1% of total.

Cadmium

With the exception of the sludge amended plot on FAZ 2, there was little cadmium present on any site, with total concentrations averaging only between 2 and 3 mg kg^{-1} where detectable. Mean EDTA-extractable Cd was also small, except on the FAZ 2 plot where total concentrations are elevated. The same pattern was repeated for $CaCl_2$ extractable Cd, which is non detectable on all plots except FAZ, although still close to analytical detection limits. However, percentage EDTA Cd recoverable from total is considerably higher on both FAZ plots, over double when compared to all other plots. KIR 1 and FAZ 1 showed similar mean concentrations of total Cd, the percentage extractable by EDTA is double on FAZ 1. This may be due to Cd on FAZ plots being derived from sewage sludge and more readily extractable in a free ion form (Alloway and Jackson 1991).

Copper

MER 2 and CRM 1 showed the highest variation in total copper levels, probably caused by each site containing highly localized elevated areas. All other sites showed considerably less variation. EDTA extraction of Cu was one of the highest for any element tested, with half of the sites extracting 40% Cu in EDTA or more. The percentage extraction exhibits a site specific pattern, all Merton sites show between 15 and 20% Cu EDTA, whilst FAZ, CRM and KIR show between 40 and 50 %, indicating a possible pH mediated difference. $CaCl_2$

extractions were poor on all sites irrespective of total concentrations with only 0.2 – 0.4% of total Cu extracted.

Nickel

Low concentrations of total Ni on all sites belie small amounts of variation in FAZ2 soil. The variation may be reflected in the lack of Ni either already in the soil, or not leaching from the areas of the plot receiving sludge. EDTA extracts little Ni at many sites, only 2 –5 %, and a maximum of 9% on KIR. Of interest however is the quantity of Ni extracted by 0.01M $CaCl_2$. This is the highest percentage extracted of total and of EDTA for any element, ranging from 0.4 – 2.7% of total Ni. It is especially noteworthy given that total soil Ni is generally low on all sites.

Lead

MER 2 exhibited the most variation in lead total concentrations, reflecting high point source areas on the plot. Other sites, with the exception of KIR, showed generally high concentrations of Pb, emphasizing the ubiquitous nature of this element and its potential to be introduced to the soil by several means, such as dry deposition in urban environments. In general more lead is extracted by EDTA than any other element tested here, with ranges between 20 and 45%. $CaCl_2$ extractions are very low given high total values, never exceeding a maximum of 0.4mg kg^{-1} when total values may be 5000 times this concentration.

Zinc

Variability of zinc was low on both FAZ sites when compared to MER 2 and 3 where variability was higher. EDTA extraction shows no overall pattern and is generally between 10 to 20% of total. Overall $CaCl_2$ extractable levels were also low given elevated total concentrations although on sites such as FAZ 2 and CRM 1 this increased to 2.8 and 1.2% respectively, possibly due to lower pH values at these sites.

Soil hotspot variability – Overview and conclusions

Considerable variability in soil parameters exists, even when addressing elevated hotspot areas of similar metal concentrations as defined by the mapping techniques in Chapter 4. This procedure for defining and experimenting on hotspots is not completely effective. On several plots, high standard deviations or confidence intervals, indicate large variations within previously identified hotspots. This reinforces arguments made earlier on the variable nature of brownfield sites, and although mapping techniques can be used to better locate and treat hotspots there must be an acceptance of variability when working on such sites. Although not tested statistically observations show sites with artificial amendments that have been incorporated homogeneously over time such as FAZ 1 tend to show the least spatial variation (Fig 5.1.1).

The need for more accurate methods of assessing soil metal bioavailability in the context of ecological risk management is well documented (Dickinson *et al.* 2000; Environment Agency 2003; Nolan *et al.* 2003). However, as discussed in Chapter 1, there is no singular recognized methodology for this and a suite of techniques have been postulated showing different responses to different metals in different field situations, such as pH and soil type. The use of $CaCl_2$ and EDTA, two common alternatives to total or pseudo-total metal testing by strong acid digest, was intended to see if a quick and reproducible test could be used to assess a variety of brownfield soils and contaminants. The ultimate goal is to use extractants to predict phyto or ecotoxicity and uptake of metals into plants or other ecological receptors. These concepts are discussed and tested later in this chapter.

EDTA produced favorable recoveries for copper and lead at all sites, and to some extent for cadmium, but only on sites where Cd concentrations were elevated, such as FAZ. For these elements a distinction could be made between plots on Merton Bank, where percentage recoveries of total were lower, compared to other sites. This was thought to be pH related (all MER sites are pH 7 or higher), but FAZ 1 also has a pH of over 7 and exhibits one of the best recoveries. EDTA recoveries for Ni and As are poor, especially for As where total concentrations were significantly elevated. Changes in site type or total metal present may strongly affect recoveries using EDTA. Pulford and Watson (2003) used EDTA to extract metals from a site that had received sewage sludge for over 50 years (Cd 49.6%, Cu 51.7%, Ni 42.0%, Pb 30.9%, Zn 48.7%, pH 6.3). These percentages extractable by EDTA from total were broadly in line with the maximum ranges achieved in this study, with the exception of Ni and Zn, which were higher in the sludge based soil.

The use of $CaCl_2$ as an extractant to simulate the bioavailability of heavy metals is strongly advocated in several sources as it matches soil solution with respect to pH, concentration and composition (Houba *et al.* 1996 ; Novozamsky *et al.* 1993) and is set to become a standard technique in some parts of Europe (Pueyo *et al.* 2003). Since one of the main mediating factors for actual plant availability of metals in soils is pH the use of a neutral buffer solution is well warranted, as opposed to strong acid digests. In this experiment $CaCl_2$ extractable Cu is between 0.1 – 0.3% of total, $CaCl_2$ percentage recoveries of total digest were also recorded by Pulford and Watson (2003), recording 0.14% for Cu. Other comparisons between these two experiments can be made: Ni ranges from 0.4 – 2.7% in Pulford's study, compared to 3.3% in the present study. On sludge amended FAZ plots, where Cd was present in elevated levels we found 1.2 – 3.5% $CaCl_2$ compared to 2.4%. Zn was more variable 0.1 – 2.8% compared to 0.63%. Lead was non detectable in both experiments.

Overall, $CaCl_2$ exhibited very low recoveries for elements such as Pb, even when high total concentrations are present. This is not unusual as it is known that Pb is extremely unavailable under natural soil conditions, which the $CaCl_2$ extractant is intended to mimic. However, although it may be more accurate indicator of natural soil conditions and possibly bioavailability, it is not of use unless empirical figures can be generated above analytical method detection levels to use in risk management models and calculations. Problems were encountered with cadmium concentrations, which shows considerable uptake by some trees even at very low soil concentrations that may not be detected by a $CaCl_2$ extract. Issues with $CaCl_2$ recoveries at low soil concentrations have been reported in other studies examining soil metal and tree interactions, specifically causing problems with statistical analysis due to low or non detectable Cd concentrations (Laureysens *et al.* 2004). Houba *et al.* (1996) noted that more sensitive analytical methods would be necessary if using $CaCl_2$. $CaCl_2$ may not be the most appropriate extractant for low level contaminated brownfield sites unless sufficiently sensitive analytical instruments can be used.

5.2 Principal Component Analysis

Principal Component Analysis (PCA) is a useful tool to examine large, multi variant data sets. Comparable studies in this field have used PCA techniques for handling large amount of varied environmental data (Watson *et al.* 2003; Laureysens *et al.* 2004). PCA has been employed in this study to assess the relative and importance of analyzed parameters and their relationships to each other, with a view to examining any significant data in additional quantitative detail. Analyses have been conducted with species data pooled from all sites. Each parameter is represented by an arrow that extends through the origin of the bi-plot, the length of each arrow is proportionate to the importance in locating species on the graph. The grouping of the species can be assessed for significance by their perpendicular relationship to the arrows. Data were analysed using CANOCO for Windows v4.

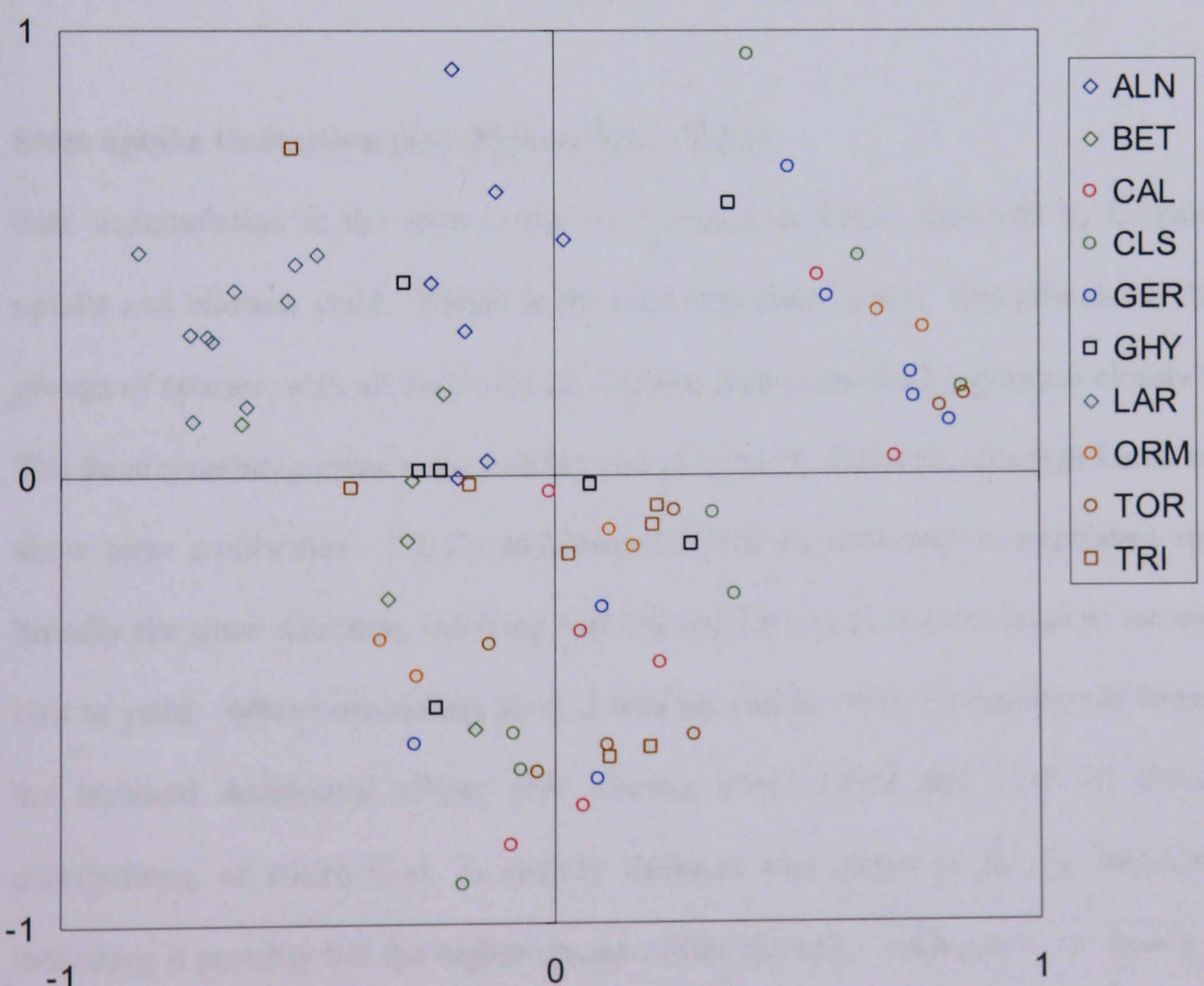


Figure 5.2.1. CANOCO bi-plot of individual species data points.

Fig 5.2.1 shows a bi-plot of each individual measured data point, indicating the need to reduce the complexity of the plot for visual clarity. The position of each of the species on the plots can be averaged using the individual positions representing each measured tree. When using this method it is important to take into consideration the distribution of the data points around the mean value, this is especially important given the pooling of species or taxa data across sites and possible variation. It is therefore desirable to plot bivariate standard deviational (SD) ellipses for axes 1 and 2, associated with the averaging of each species or taxa. This methodology was given by Ricklefs and Nealan (1998) but adapted for use in MS Excel by Le Duc *et al.* (in press), and recently used in Milligan *et al.* (2004), which was the method utilized to produce the ellipses shown here. These are presented, for clarity, on the following page for each main ordination plot for each species group, and are directly comparable to the ordination plots representing the measured parameters.

Stem uptake Ordination plot (Figures 5.2.2 / 5.2.3)

Zinc accumulation in the stem is the most important factor, followed by Cadmium stem uptake and biomass yield. Nickel is the least important factor. The plot shows distinctive groups of species, with all *Salix* and all *Populus* taxa respectively, grouped closely together. The three remaining species are suitably and predictably different, although Larch and Alder show some similarities. Cd, Zn and biomass yield are indicated as significant and follow broadly the same direction, inferring that Cd and Zn can be accumulated in the stem at no cost to yield. When considering *Salix,* 3 taxa are similar when comparing the orientation of the standard deviational ellipse (SD ellipse); CAL, ORM and TOR all show similar distributions, of which CAL is slightly different and closer to the Zn and Cd arrows, indicating it possibly has the higher uptake of this element. GER and CLS show a different SD ellipse. This is possibly due to their affinity to accumulate copper in the stem, moving the points in that direction. Interestingly the Cu stem line is running opposite to the Cd and Zn lines. This is possibly due to the high affinity of LAR and ALN for Cu in the stem. Although other willows do take up Cu, ALN and LAR only take up Cu. This is what may be keeping the *Salix* points closer to the origin. It is somewhat misleading, as it could be interpreted that willows do not take up Cu, data presented in earlier sections show that they do. Poplars are grouped together near the origin, indication that no one factor is significantly more important than the other. Pb and Ni are predictably insignificant.

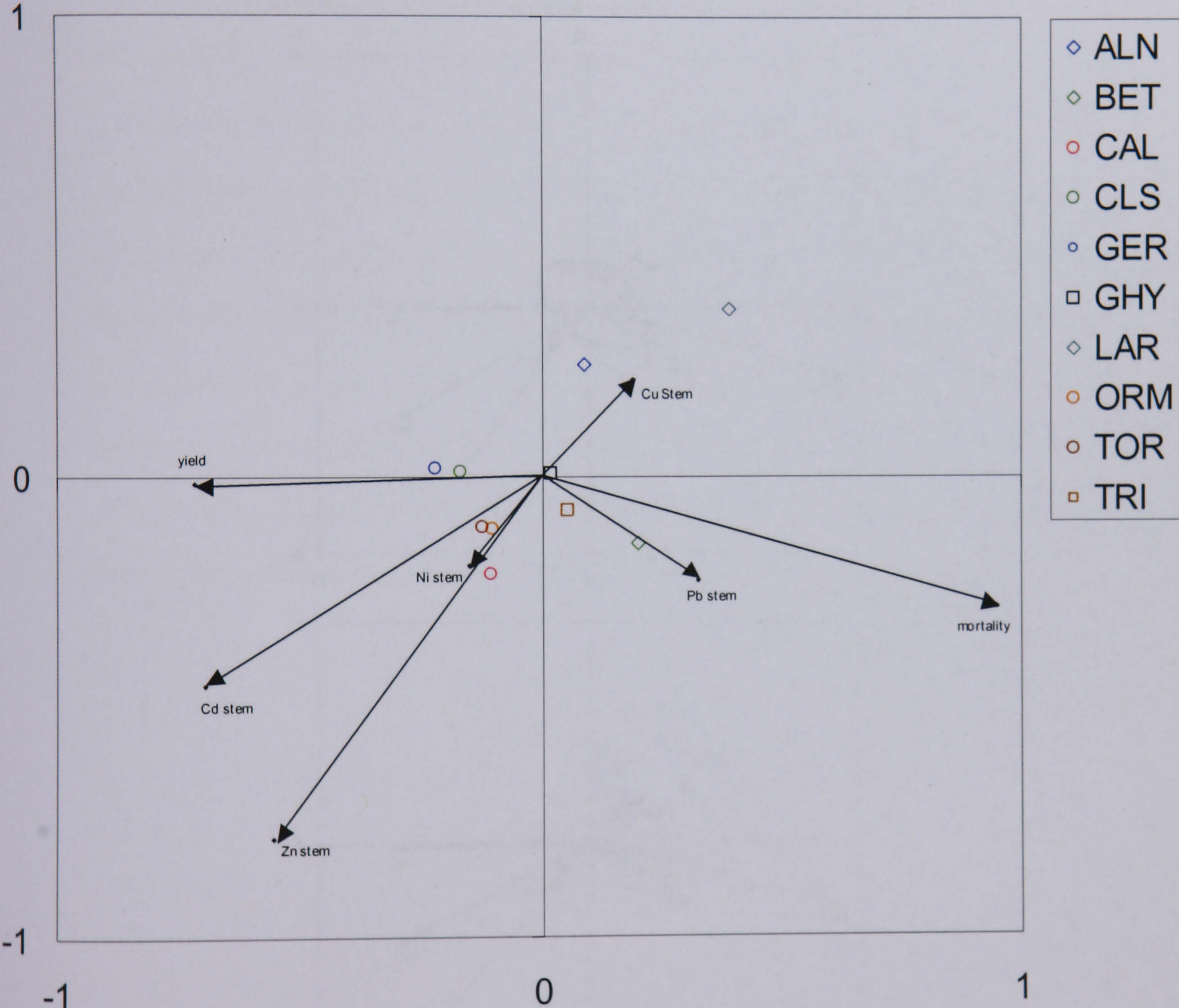


Figure 5.2.2. Stem uptake (year 3) CANOCO ordination bi-plot for all species.

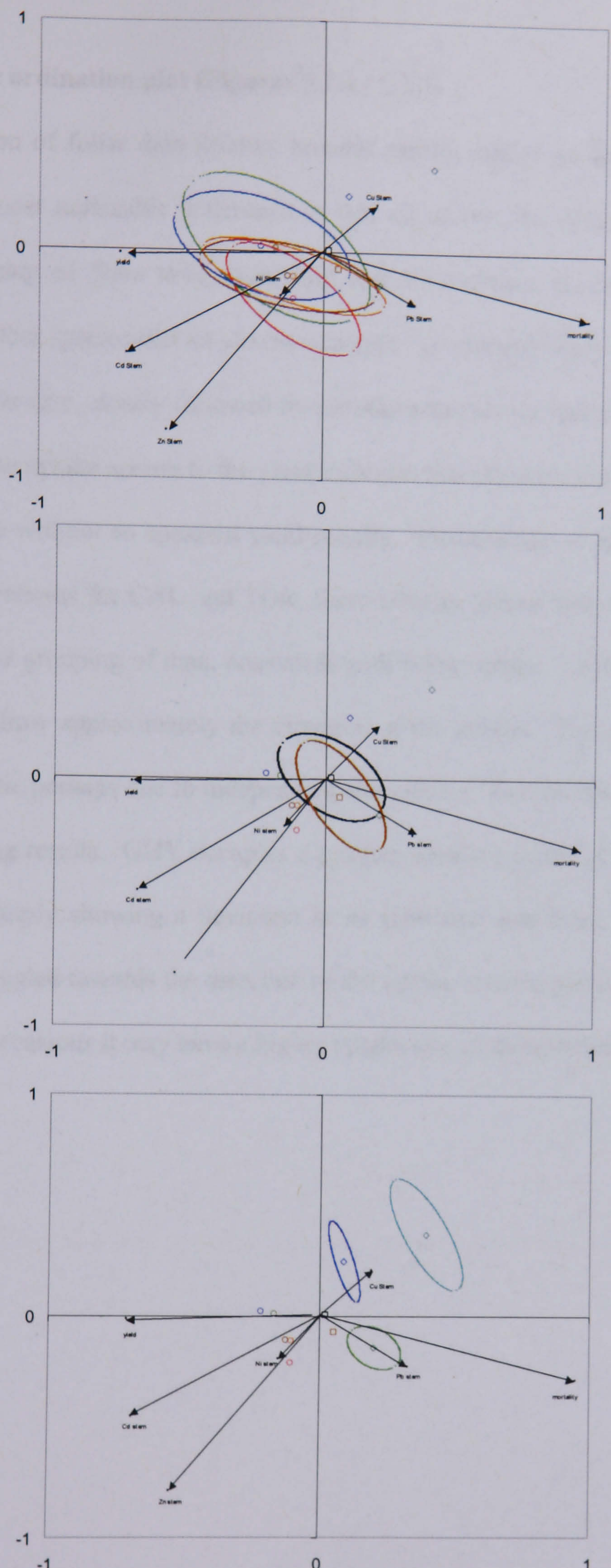


Figure 5.2.3. Standard deviational ellipses for Stem uptake PCA ordination bi-plot (figure 5.2.2) showing Salix (top), Populus (middle) and other species (bottom). The colour of the ellipses represents associated species as shown in Figure 5.2.2.

Foliar uptake ordination plot (Figures 5.2.4 / 5.2.5)

The distribution of foliar data follows broadly similar patters to the stem analysis (Figure 5.2.2). The most noticeable difference is that all arrows for uptake run in a tight group though the group of *Salix* taxa, indicating that all elements are taken up by these taxa, compared to other species that sit almost opposite, for example ALN and LAR. Similarly to stem results it is zinc, closely followed by cadmium that are the most important factors. The proximity of the uptake arrows to the yield indicates that metals in these taxa can be taken up into the foliage without an apparent yield penalty. Examination of the SD ellipses for *Salix* show similar patterns for CAL and TOR, these ellipses follow almost precisely the arrows, showing a good grouping of data, consistent with foliar uptake. CLS and GER show wider ellipses and follow approximately the direction of the arrows. This could indicate a wider variation of data, perhaps due to independent site effects. The SD ellipses for *Populus* show some interesting results. GHY occupies a position almost exactly at the origin, with a very circular SD, simply showing a deviation in no particular direction. TRI however has an ellipse that is angled towards the direction of the uptake arrows, particularly Zinc, indicating that, on some occasions it may have a higher uptake rate of this element.

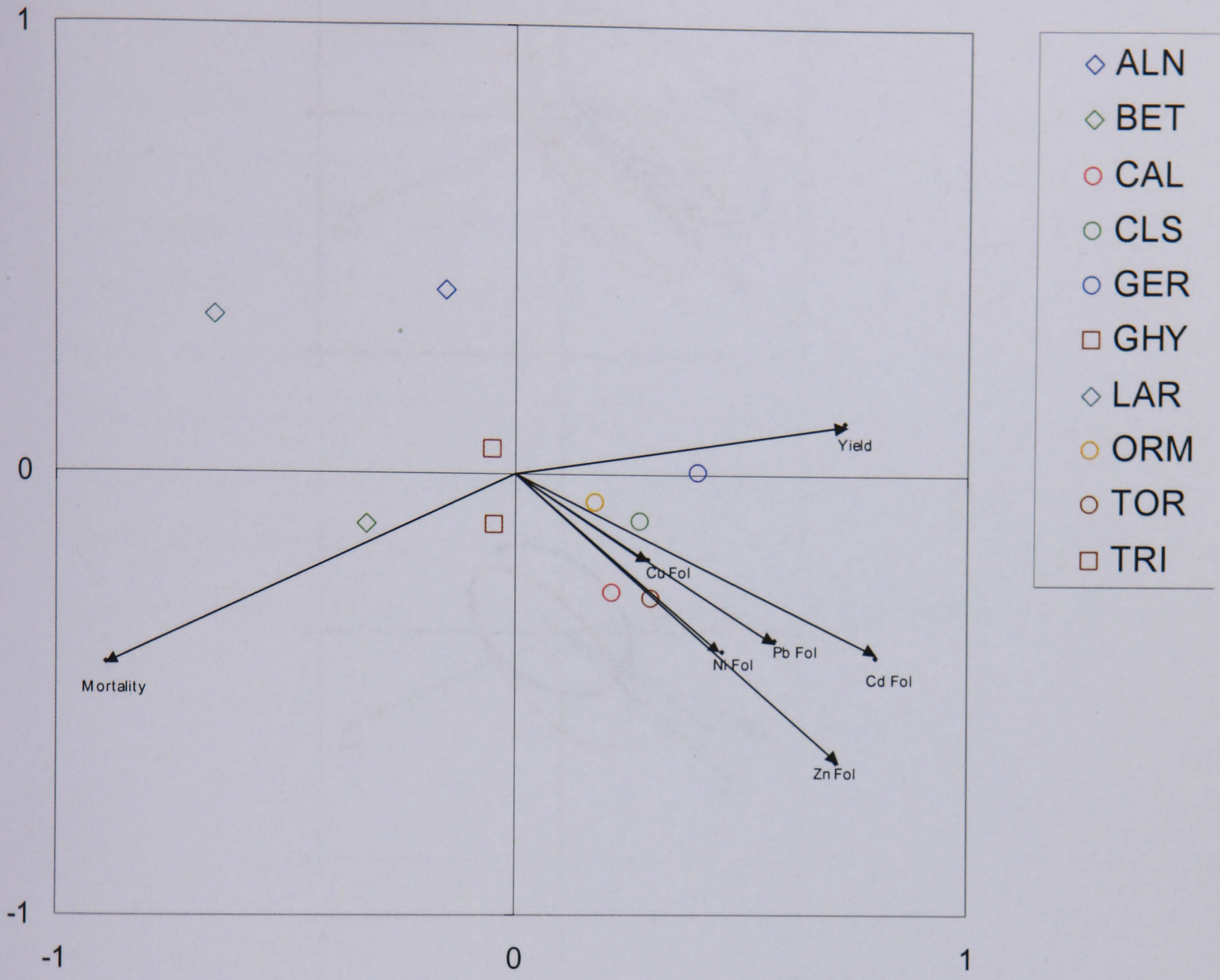


Figure 5.2.4. Foliar uptake (year 3) CANOCO ordination bi-plot for all species

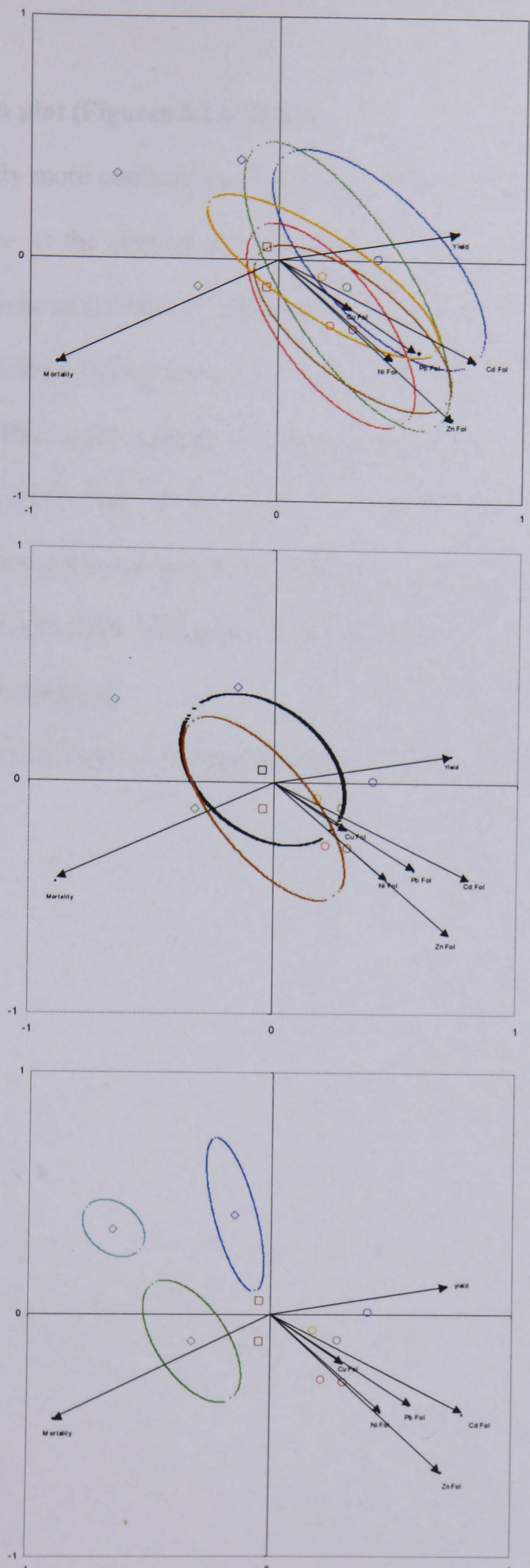


Figure 5.2.5. Standard deviational ellipses for Foliar uptake PCA ordination bi-plot (figure 5.2.4) showing Salix (top), Populus (middle) and other species (bottom). The colour of the ellipses represents associated species as shown in Figure 5.2.4.

EDTA ordination plot (Figures 5.2.6 / 5.2.7)

This plot is slightly more confusing as is shows the EDTA metal concentrations in the soil under each species at the start of the experiment. It may therefore be more relevant to compare the positions of the taxa to yield and mortality. Four of the *Salix* taxa are grouped close together following a general trend of Cu, Cd, Zn and Ni arrows, but more importantly the yield arrow. This could indicate that these elements can exist in the soil with no yield penalty for these species, and yield is one of the most important factors in their positioning. The As EDTA arrow is located broadly towards the mortality arrow, certainly more so when compared to other elements, indicating the concentrations of this element in soil may be having an effect on mortality.

The SD ellipses are all large, reflecting the range of soil data and variability from different sites.

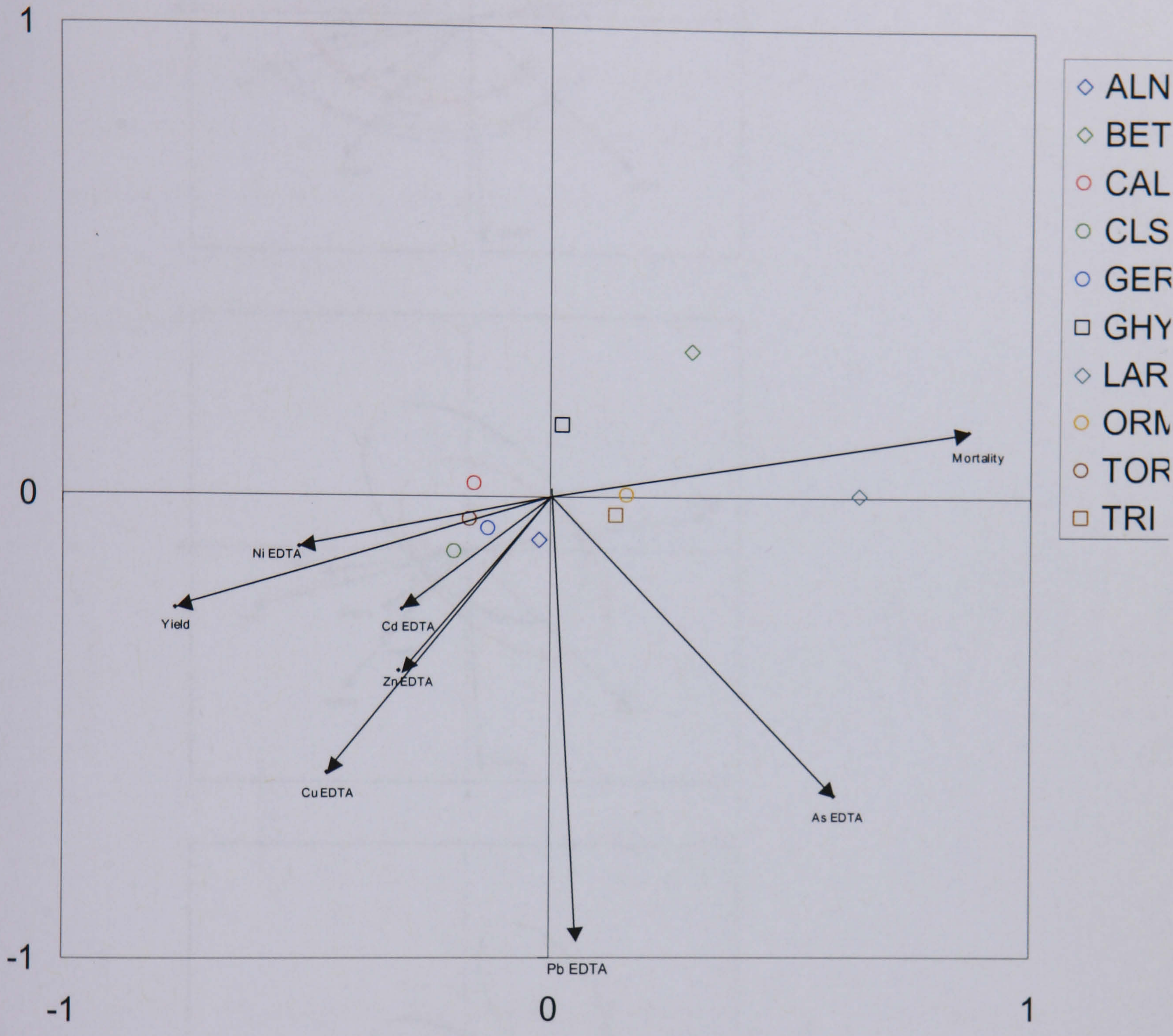


Figure 5.2.6. Soil EDTA extractable metal concentration (year 1) CANOCO ordination bi-plot for all species.

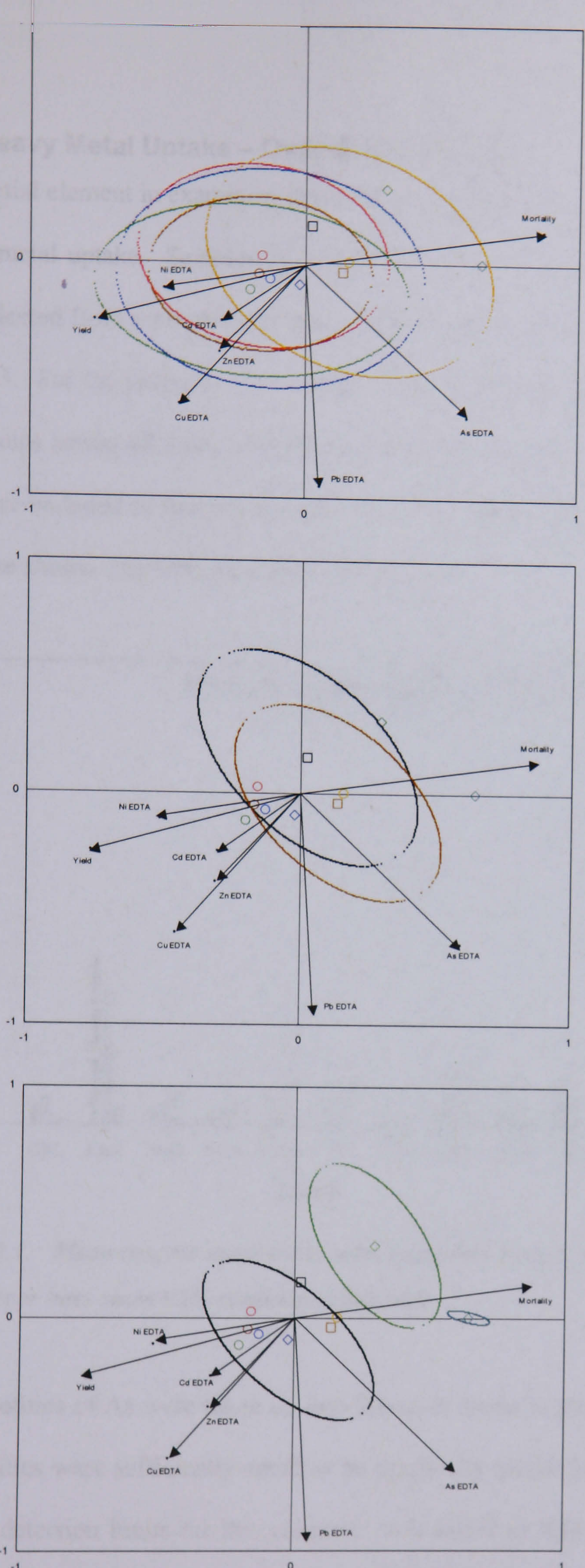


Figure 5.2.7. Standard deviational ellipses for EDTA soil concentration PCA ordination bi-plot (figure 5.2.6) showing Salix (top), Populus (middle) and other species (bottom). The colour of the ellipses represents associated species as shown in Figure 5.2.6.

5.3 Heavy Metal Uptake – Overall Assessment

An essential element in examining prospects for phytoremediation is obtaining accurate field data on metal uptake. Samples of foliage (for Years 2 and 3) and stem (for Year 3 only) were collected from replicate trees surrounding hotspot soil sampling points, as described in Chapter 3. For the purposes of assessing broad patterns of uptake this data was pooled for each species across all sites, with the exception of Arsenic where only species on Merton Bank were included as this was the only site with significant concentrations of soil As. All figures are shown with 95% confidence intervals.

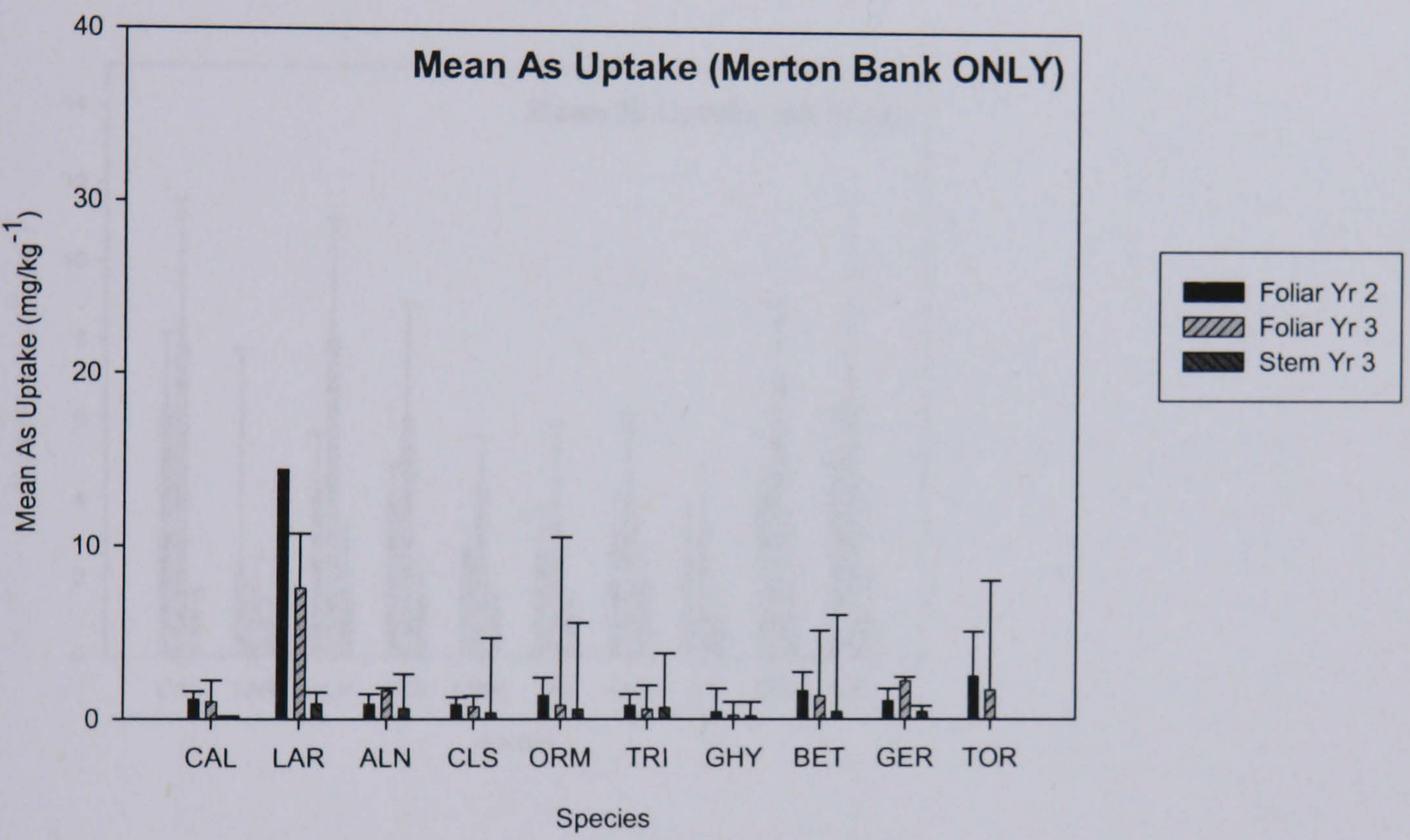


Figure 5.2.1. Mean arsenic uptake into stem and foliar tissues by trees at Merton Bank (MER). Error bars show 95% confidence intervals

Arsenic

Small quantities of As were taken up into leaves or stems in almost all species (Fig. 5.2.1). The quantities were sufficiently small to be practically insignificant, especially considering analytical detection limits for this element. It is therefore difficult to distinguish any true pattern of translocation. Larch was a notable exception, accumulating significant concentrations (5 to 10 times that of other species) of As into its foliage despite high variation through a low number of samples during Year 2. The stem of larch however, accumulated no more As than any other species.

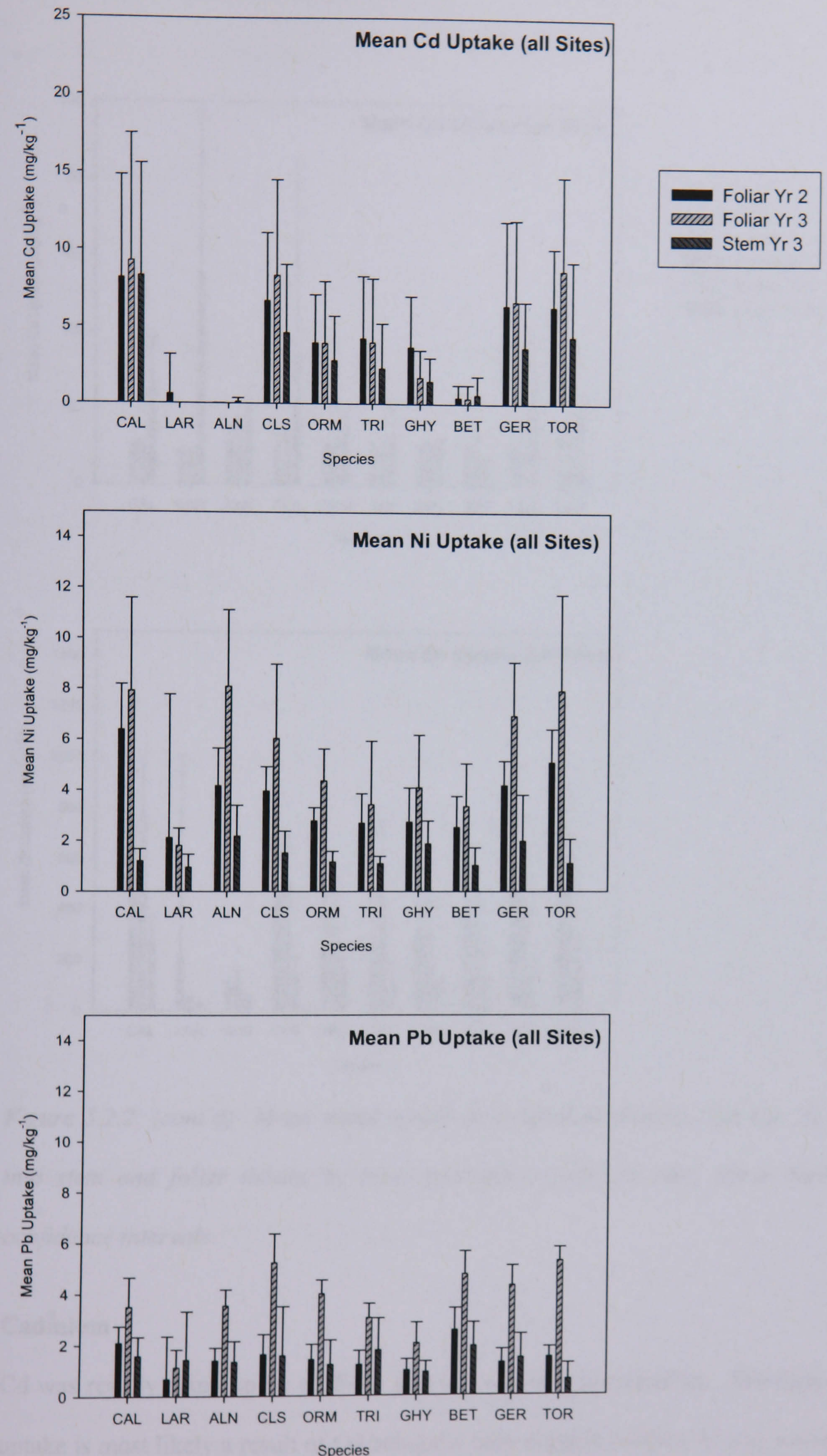
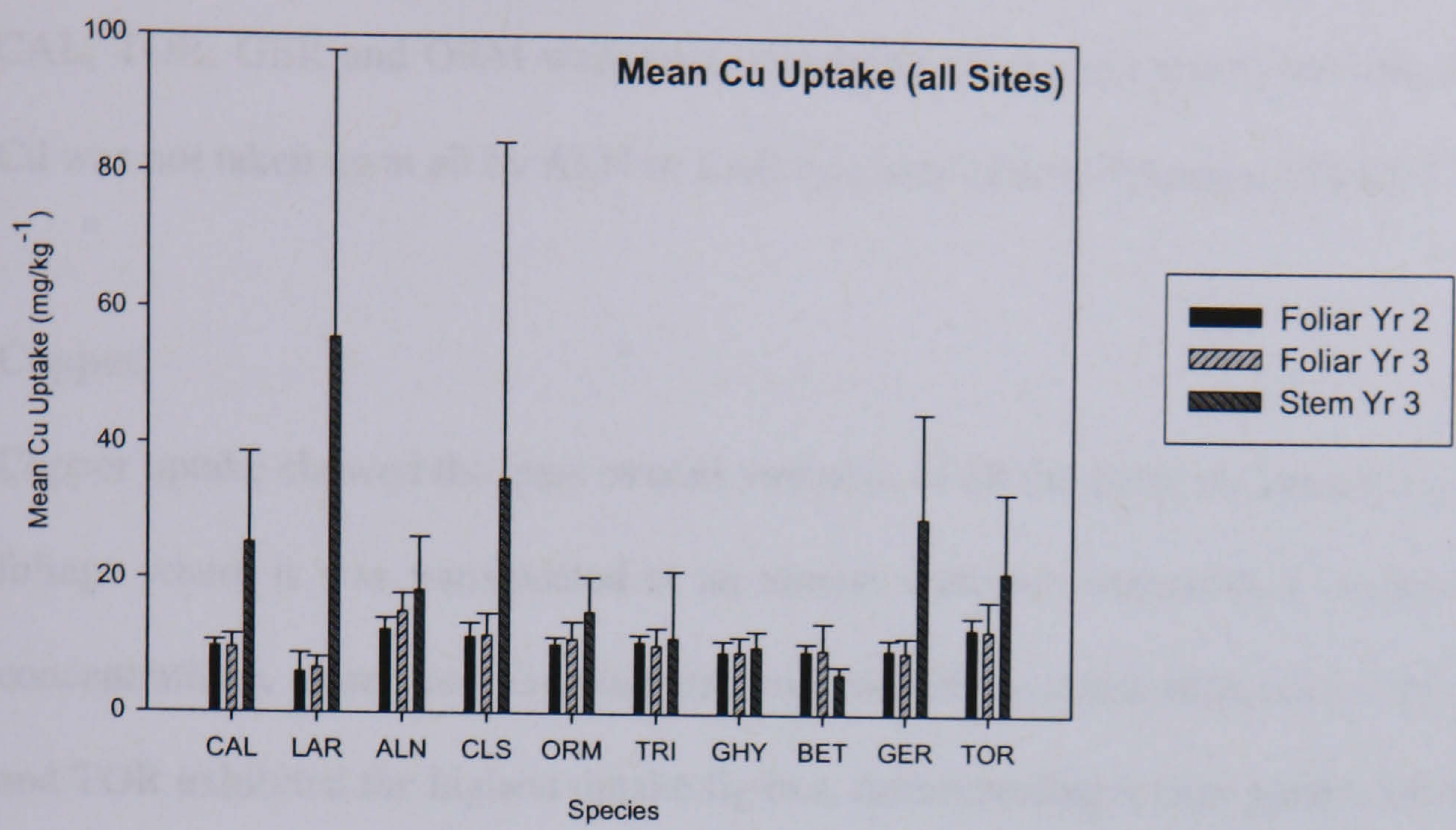


Figure 5.2.2. Mean metal uptake by individual element (Cd, Cu, Ni, Pb and Zn) into stem and foliar tissues by trees averaged across all sites . Error bars show 95% confidence intervals



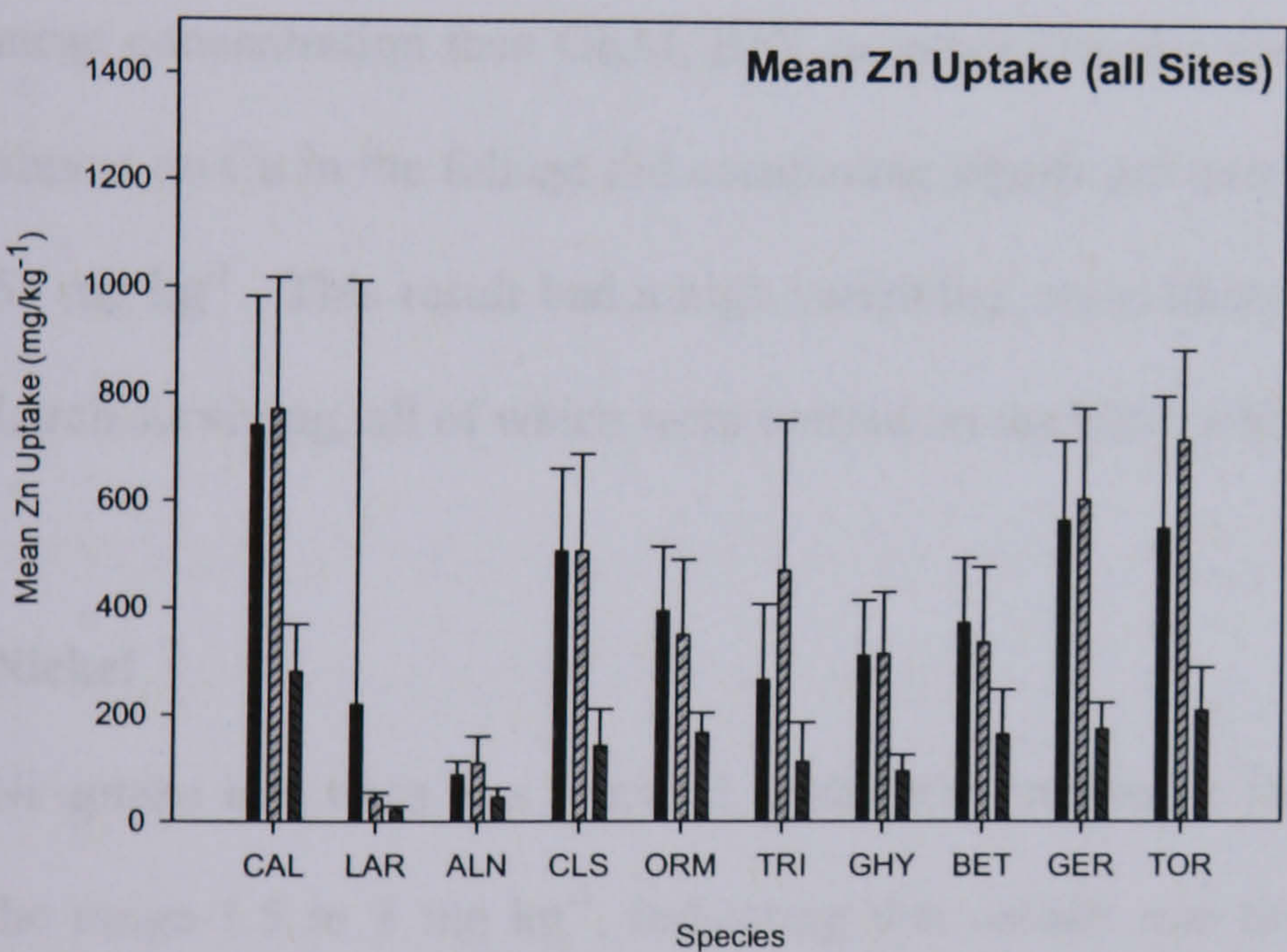


Figure 5.2.2. (cont'd) Mean metal uptake by individual element (Cd, Cu, Ni, Pb and Zn) into stem and foliar tissues by trees averaged across all sites. Error bars show 95% confidence intervals

Cadmium

Cd was readily taken up by all *Salix* taxa in varying concentrations. The large variation in uptake is most likely a result of Cd being the only element besides As and possibly Ni to not be found at all on some sites, compared to elements such as Cu, Zn and Pb that were represented on all sites to some degree. This high variance makes it difficult at a macro scale to detect significant patterns of distribution within the tree, although in 4 of 5 *Salix* taxa more Cd is located the leaves than the stem, albeit that differences are relatively small. CAL, TOR, GER and ORM were taxa containing the highest concentrations of Cd overall. Cd was not taken up at all by ALN or LAR and only in small quantities by BET.

Copper

Copper uptake showed the least overall variation of all the elements measured, especially in foliage where it was translocated at an almost uniform concentration, irrespective of soil concentrations. Increased variation occurred within the stem; *Salix* taxa, CAL, CLS, GER and TOR exhibited the highest uptake figures, demonstrating a clear pattern of concentration of Cu in the stem, between 20 – 35 mg kg^{-1}. ALN transferred Cu into the stem at a higher mean concentration than ORM, BET or either *Populus* taxa. Larch, although accumulating almost no Cu in the foliage did accumulate significant quantities into the stem, an average of 55 mg kg^{-1}. This result had a high variability, most likely as a result of a small number of Larch surviving, all of which were located on the Merton Bank site.

Nickel

Ni uptake into trees was minimal, with little variance in the stem uptake figures that are in the range 1.5 to 3 mg kg^{-1}, indicating that uptake was low overall. More variation was observed in Ni that had been transferred to foliage. Due to this variation it is difficult to distinguish any obvious pattern, but the general trend shows CAL, CLS, GER, TOR and ALN accumulating elevated Ni in foliage.

Lead

Very small quantities of lead were translocated into plant biomass and all mean stem values were between 0 and 2 mg kg^{-1}. Foliar concentrations, although slightly higher were only in the range 2 to 5 mg kg^{-1}. Variation is low overall reflecting a general trend for low Pb uptake on all sites. Very low detectable concentrations make this data of questionable significance, based on analytical detection limits and background concentrations or external contamination. The very low concentrations mean that phytoextraction would not seem a realistic option at these sites in its present context.

Zinc

Considerable quantities of zinc were accumulated by all species, the highest for any element under study. A clear overall pattern of higher accumulation in foliage than stem, by up to two thirds, is observed for all species or taxa tested. The general exceptions to this were ALN and LAR with little Zn uptake into foliage or stem. Variation was increased in foliage uptake than stem uptake.

Overview of heavy metal uptake

The pooling of data across sites has been used to present an overview of the uptake potential of various species grown on brownfield sites. Strong uptake patterns are shown with the elements Cd, Cu and Zn and poor uptake is exhibited with As, Ni and Pb. These results are not unexpected. Previous studies have shown Pb to be highly unavailable to plants (Huang *et al.* 1997; Schmidt 2003) and As forms stable compounds within soil (Pongratz 1998; Wasay *et al.* 2000).

Ni has been shown to accumulate in trees and plant species, but in experiments where soil concentrations of Ni have been considerably higher then those present in this study (Pulford *et al.* 2002), or where artificial chelation had been used to improve Ni uptake (Panwar *et al.* 2002).

Mean uptake figures do not consider the concentrations of individual elements present in the soil, for example Cd uptake may appear low, but is based on very low soil concentrations. Conversely Pb uptake is low in plants but soil concentrations are generally much higher. This will be further explored in subsequent sections and chapters (Chapter 7).

Non-coppice species

There are exceptions from the observations made above, many of which relate to the non coppice species tested. Larch accumulates almost 10 times more Arsenic than any other species into its foliage with a range of 8-14 mg kg^{-1}. Although this concentration is still too low to be useful for phytoextraction, it is significant in the context of this experiment as it was the only species tested to extract this concentration of As. Larch also exhibited the highest Cu uptake into the stem with a range of 18 – 127 mg $kg^{-1.}$ Unfortunately the only Larch tested were on Merton Bank as the species suffered increased mortalities during establishment on all other sites. It was therefore not possible to examine if this level of uptake is repeated on other sites. Elevated concentrations of Cu in other *Larix* species have been reported as part of macronutrient studies on non-contaminated woodlands (Alriksson and Eriksson 2001) and when used as biogeochemical indicators of pollution in the alpine environment (Orlandi *et al.* 2002). No reference could be found to use in phytoremediation schemes, although Alriksson and Eriksson (2001) recorded increased concentrations of Cu in *Picea abies*. It is likely that Cu is more mobile in stems of coniferous trees due to the way in which it is transported and stored within the stem.

Alnus translocated Cu into the stem at a higher mean concentration than ORM, BET or either *Populus* taxa. LAR and ALN translolcated the lowest concentrations of Zn, perhaps due to a preference for Cu, but at a lower uptake rate. LAR, ALN and BET all actively exclude Cd from stem and foliage at the soil concentrations found in this experiment.

Populus

Populus has not received the same level of attention as *Salix* have when considering their use in the phytoremediation of heavy metals. There is however, a continuing interest in the use of poplars to degrade organic contaminants (Burken and Schnoor 1998) such as high explosives (Yoon *et al.* 2002); TCE (Newman *et al.* 1997; Shang and Gordon 2002);

Petrochemicals (Rentz *et al.* 2003) and atrazine (Burken and Schnoor 1997). In this experiment *Populus*, like *Salix* showed poor uptake of As, Ni, Pb and Cu into foliage. Cd and Zn are accumulated to some extent and were higher in TRI than GHY. Zinc was significantly elevated in foliage of TRI with a high variance suggesting differences between sites.

Other studies have shown broadly similar patterns. Moffat *et al.* (2001) tested several *Populus* taxa, including TRI, using experimental treatments of sewage sludge and irrigation and concluded average stem concentrations for TRI were Cd, 6.6, Zn, 51, Cu, 5.5 (all values mg kg^{-1}), which are within the ranges found in the present study. Foliar data was not presented but Zn and Cu were reported to be significantly elevated.

Sebastiani *et al.* (2004) conducted pot trials using 2 *Populus* taxa amended with organic industrial waste high in Zn and Cr. After 1 year Zn concentrations were 300 mg kg^{-1} in foliage, 80mg kg^{-1} in stem and 100 mg kg^{-1} in roots. Cu and Cr were not significantly elevated, although Cu uptake into roots was 5-6 times higher than maximum stem uptake. Di Baccio *et al.* (2003) conducted similar experiments, in pots spiked with zinc solution. At the maximum Zn concentration used (1000μM) leaves accumulated 160 mg kg^{-1}, stem 60 mg kg^{-1} and 90 mg kg^{-1} in roots. The two latter experiments focused on different taxa not used in the current experiment, specifically *Populus* 'Eridano' (*Populus deltoides x maximowiczii*) and I-214 (*P. x euramericana*). By comparison uptake rates of Zn are higher in this study than others cited in the literature, despite soil concentrations being generally lower than those used elsewhere in organic or artificial amendments. All studies concur that *Populus* only accumulates Zn at consistently elevated levels. Although other studies show it is possible for Poplar to accumulate Cd (Robinson *et al.* 2000) it does not seem to match the concentrations accumulated by *Salix*. Using chelating materials to improve Cd availability to poplars had unwanted side effects such as growth reduction and leaf abscission (Robinson *et al.* 2000).

Salix

Uptake of heavy metal elements into above ground biomass by *Salix* has been well researched (see Chapter 1). Recent publications have concentrated on Cd and Zn as these elements have been shown to be consistently translocated by *Salix*. Similar patterns were apparent in this study, with all *Salix* taxa able to accumulate Zn and Cd, and in some cases Cu.

Of these elements, Cd can be considered most hazardous from an ecological and human perspective (DEFRA and Environment Agency 2002f). CAL, TOR, GER and ORM were taxa containing the highest concentrations of Cd, but variations in uptake seem to indicate that taxa will accumulate more Cd if it is present at elevated soil concentrations. This concept is examined later in the chapter. *Salix* have shown the ability to accumulate Cd from soils containing relatively low concentrations. This trait can be examined by deriving and comparing concentrations factors. Concentration factors (CF) were calculated as a ratio of soil EDTA available Cd to concentrations measured in above ground biomass constituents (foliar and stem), therefore showing the factor at which Cd can accumulate in the biomass greater than in the soil. Table 5.2.1 shows the average concentration factors and rankings in the stem and foliage of *Salix* taxa pooled across all sites.

CAL followed by GER are able to concentrate the most Cd into the stem, the other 3 taxa show similar factors between 4.4 and 4.8. Other studies have examined concentration factors of soil to biomass for Cd in various *Salix* taxa, although many used soil data based on total acid extraction, not EDTA. Felix (1997) found a transfer coefficient of 3.4 for *S. viminalis*, and Punshon and Dickinson (1997), frequently observed concentration factors (above-ground tissues : soil) above 5 in *S. caprea*. Pulford *et al.* (2002) found stem Cd concentrations in 3 – year old stands of 20 clones of *Salix* that were up to 10 x higher than soil concentrations. Elevated concentrations of Cd uptake have been recorded in trees grown on highly contaminated substrates, with 125 mg kg^{-1} Cd recorded in taxa grown on mine spoil, although with substantially reduced yield (Punshon and Dickinson 1997).

Table 5.2.1. Concentration Factors (CF) for all Salix *taxa of EDTA extractable soil Cd and Stem or foliar Cd. Data are averaged for all sites.*

Taxa		CF EDTA / Stem	CF EDTA / Foliar
CAL	Min	7.18	2.20
	Max	11.18	21.00
	Average	**8.88**	**9.31**
	Std. Dev	*1.63*	*5.48*
	Rank	**1**	**3**
CLS	Min	2.13	3.04
	Max	9.59	13.36
	Average	**4.77**	**7.86**
	Std. Dev	*2.67*	*2.76*
	Rank	**4**	**4**
GER	Min	1.43	4.56
	Max	17.06	29.35
	Average	**7.74**	**13.21**
	Std. Dev	*4.37*	*6.91*
	Rank	**2**	**1**
ORM	Min	0.98	0.23
	Max	12.08	16.98
	Average	**4.85**	**5.68**
	Std. Dev	*4.99*	*4.57*
	Rank	**3**	**5**
TOR	Min	0.00	1.93
	Max	7.64	20.65
	Average	**4.43**	**10.62**
	Std. Dev	*3.34*	*4.96*
	Rank	**5**	**2**

This information would suggest that CAL and GER would be the best taxa for use in a phytoextraction program, exhibiting the greatest ability to concentrate Cd from the soil.

However, this data only presents a measure of the innate ability of individual plants to concentrate Cd within their biomass, and does not consider the amount of biomass produced, which will have a significant influence in the net Cd removed from the soil into above ground biomass. This is a factor that is frequently overlooked when assessing *Salix* potential for phytoremediation and is addressed in Chapters 6 and 7.

5.4 Relationships in metal distribution I: Stem and leaves

For taxa that accumulate elements in their above ground biomass, the partitioning and storage of these elements within the constituent parts of the tree is of considerable interest. The relative concentrations present in the leaves and stem is important as translocation into the harvestable stem may be a primary sink of metal storage if used in phytoextraction. Conversely, low stem uptake would be desirable for phytostabilisation, biomass production or ecologically sensitive areas. Concentrations in leaves are also of interest as foliage may not be harvested and falling leaves in autumn may return metals to the surface soil or wider environment.

To obtain accurate concentrations of metals in stems they must be harvested and a representative sub-sample analysed. If a clear relationship between stem and foliar uptake were found, it would be considerably quicker, less destructive and would present an easier analytical route to evaluate foliar samples and calculate stem concentrations from this data.

Initial relationships between stem and foliar uptake were conducted using Pearsons product moment correlations (Table 5.4.1) using transformed data (log x+1) for stem and corresponding foliar concentrations in Year 3, for each species, pooled over all sites and for each metal. Significant results were subjected to additional scrutiny, initially investigating r^2 values as shown in Table 5.4.2 and scatterplots on Figures 5.4.1 to 5.4.3

Table 5.4.1. Pearson's Product Moment correlation matrix for foliar and stem concentrations in Year 3. Values shown are person product moment correlation (r) and p values.

	ALN	BET	CAL	CLS	GER	GHY	ORM	TOR	TRI
Cd			0.973 <0.001**	0.955 <0.001**	0.870 <0.001**	0.894 <0.001**	0.984 <0.001**	0.973 <0.001**	0.961 <0.001**
Cu									-.886 0.19*
Ni	0.832 0.01*								
Pb	0.727 0.041*	-.843 0.017	0.791 0.019*						
Zn	0.858 0.006**		0.825 0.012*	.965 <0.001**	0.900 <0.001**				0.848 0.008**

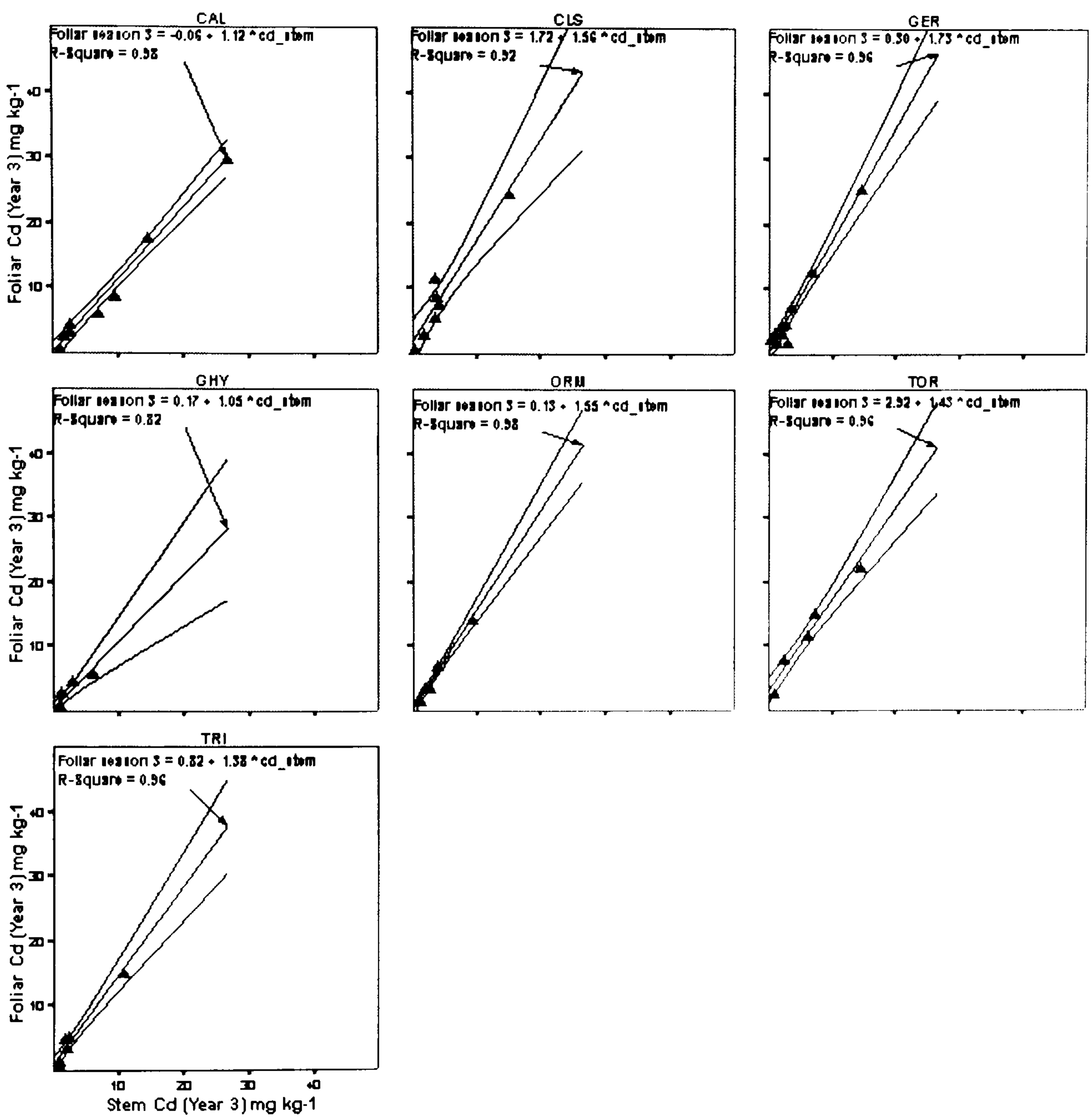
Table 5.4.2. r^2 values for significant correlations produced in Table 5.3.1 for foliar and stem metal concentrations in Year 3

	ALN	BET	CAL	CLS	GER	GHY	ORM	TOR	TRI
Cd			0.98	0.92	0.96	0.82	0.98	0.96	0.96
Cu									0.59
Ni	0.65								
Pb	0.52	0.82	0.63						
Zn	0.79		0.56	0.84	0.77				0.91

Strong correlations were observed between stem and foliar concentrations for all willow and poplar taxa with Cd; all *Salix* had r^2 values of 0.92 or higher (Table 5.4.2). Zinc was also strongly correlated, although r^2 values were more varied and showed significantly more variation within genera; *Alnus*, three *Salix* and one *Populus* showed significant r^2 values. Correlations for Cu and Ni were limited to just one taxa, with lower r^2 values. Although Pb exhibited a variety of correlations, the exceptionally low concentrations in foliage and particularly stem tissue, make the possibility of distortion of results very high.

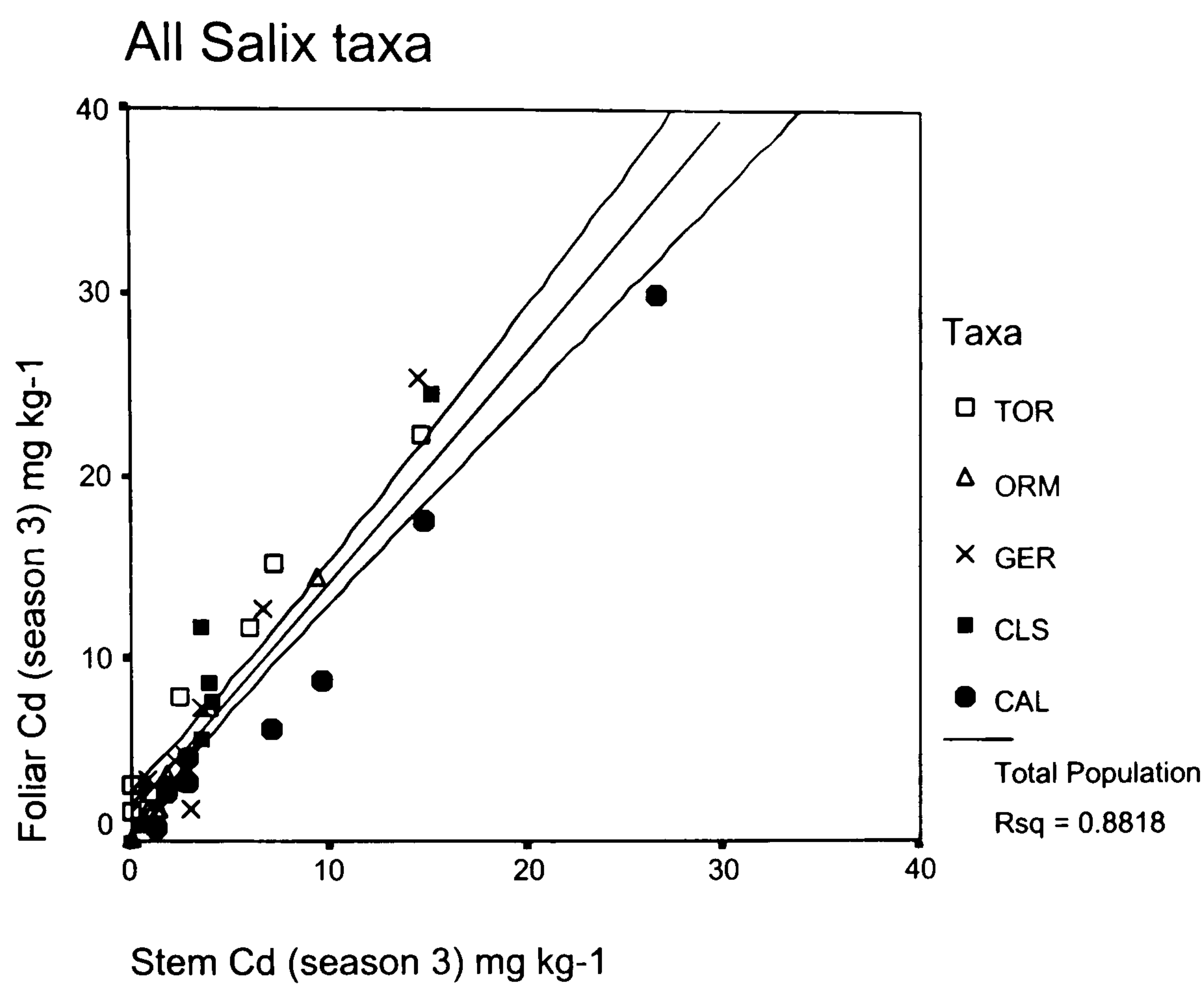
Cd and Zn were investigated in more detail with subsequent analyses of these data.

Figure 5.4.1. Cd concentration relationships between stem and foliage (p< 0.001). Lines either side of indicated extrapolated correlation line are 95% limits.



All *Salix* taxa showed a good distribution of data points from the range of sites, the majority within 95% CI's. The spread of data for *Populus* taxa GHY and TRI was not as wide, probably due to low overall Cd uptake (Figure 5.4.1). When all *Salix* taxa were pooled (Figure 5.4.2) the overall r^2 value of 0.88 showed that, despite immense variation in soil and site conditions across the range of sites used to produce the data, these taxa show a strong relationship between stem and leaf concentrations regardless of site conditions. CAL appears to accumulate the highest amounts in the stem. Although accumulating less Cd is above ground parts, *Populus* showed a similar relationship for the two taxa planted.

Figure 5.4.2. Stem versus foliar concentrations of Cd when (a) Salix and (b) Populus are separately grouped within their own genus



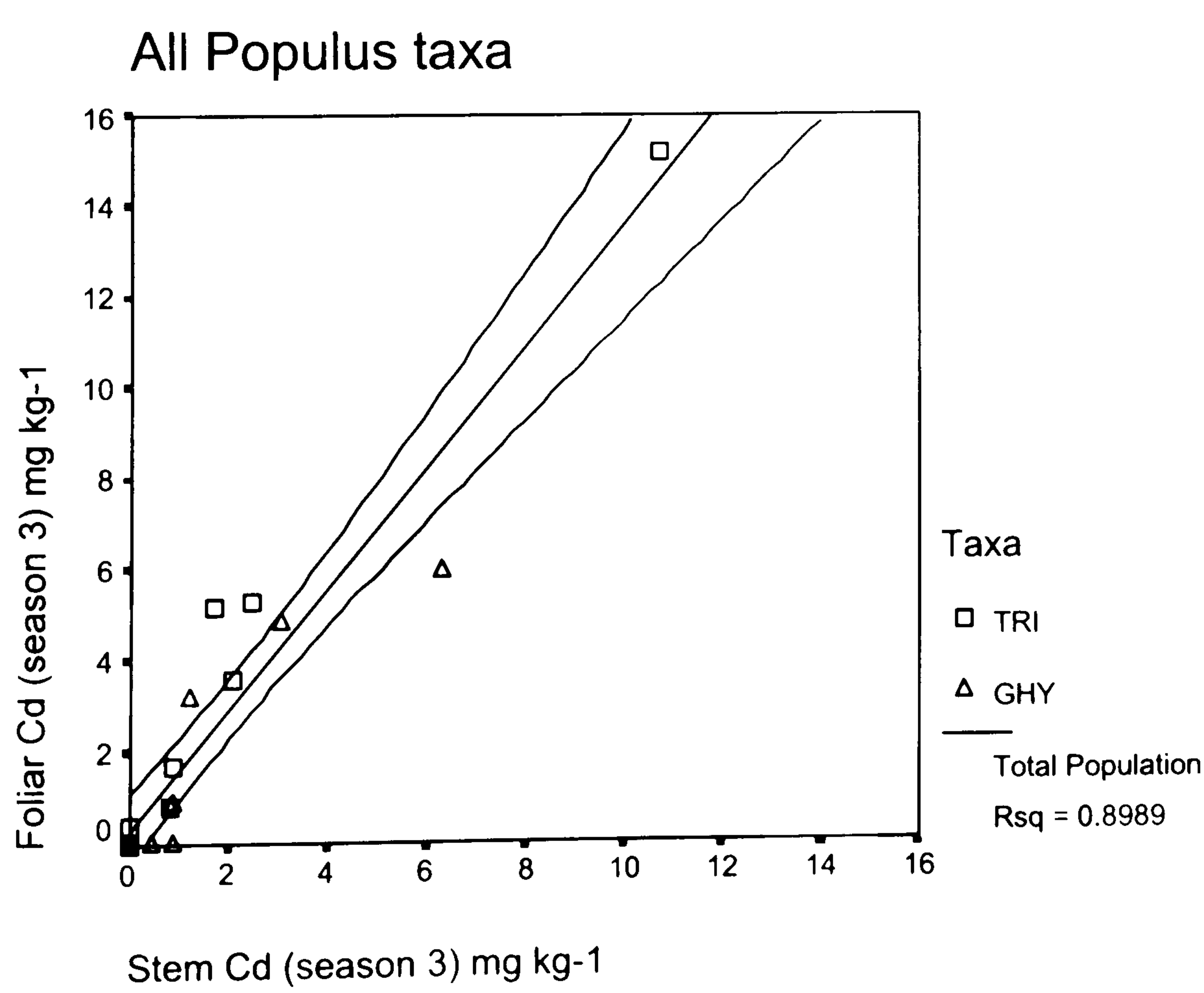
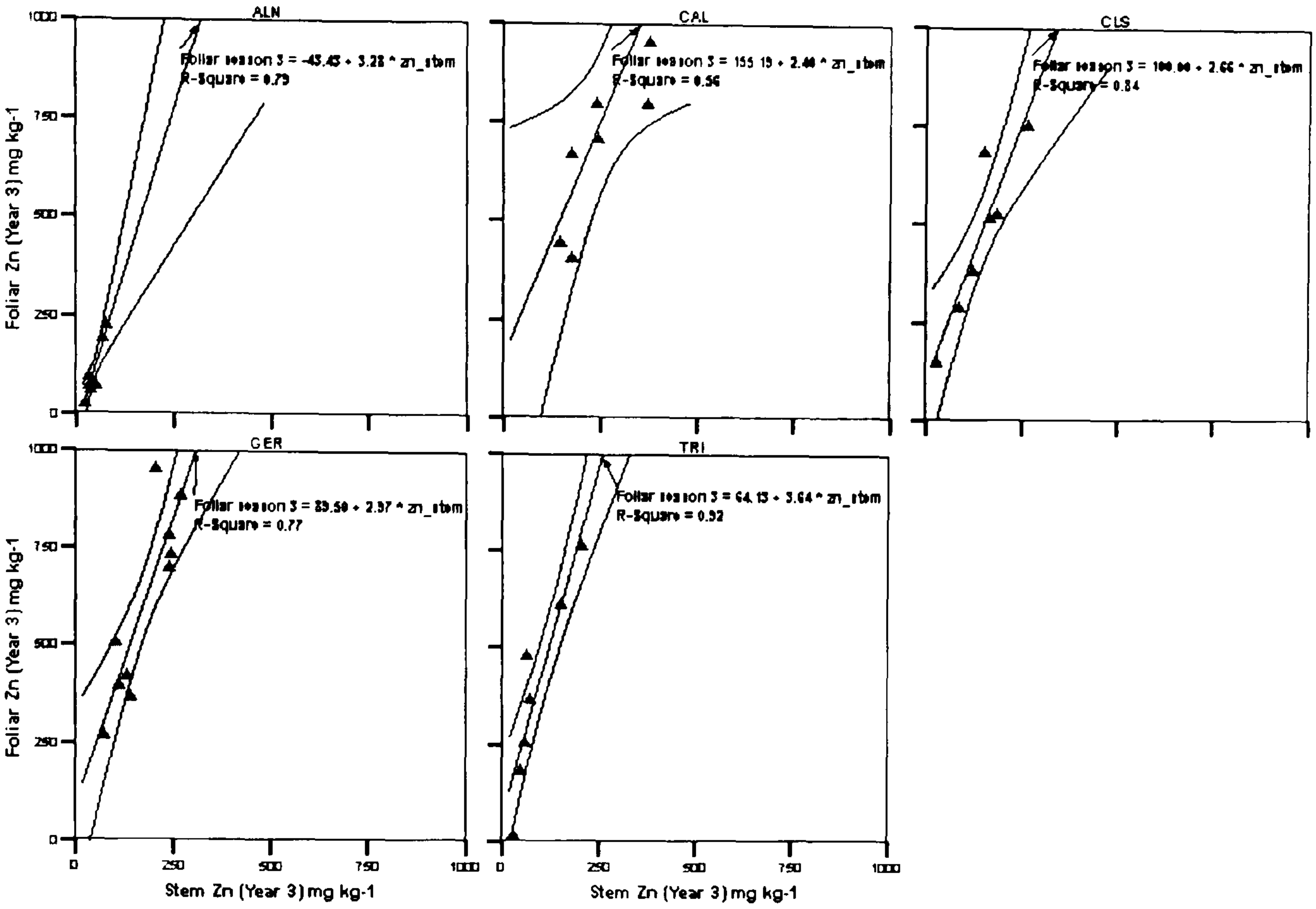


Figure 5.4.3. Zn concentration relationships between stem and foliage (p< 0.05). Lines either side of indicated extrapolated correlation line are 95% limits.



Zinc does not show the same levels of correlation between stem and foliage as found with cadmium. ALN has a high r^2 value, but over a narrow data range. CLS and GER and TRI do show a strong correlation and a good distribution of data at a range of values. Zn does not however, show the same correlation within the whole genus as was the case for Cd (Fig 5.4.2) and data is not presented here.

Stem and Foliar metal relationship Overview

There is evidence that variation in uptake and partitioning is present in differing species and for some species and metal interactions a generalized prediction can be made on stem metals in comparison to foliar uptake.

Clearly there is variation in the ratio over time (seasons and years) that is not reflected in the current analysis; it is likely that more metals would accumulate within the stem with time, changing the metal ratio between foliage and stem. Several studies have reported that metal concentrations change with vertical distribution within the stem (Sander and Ericsson 1998), although a careful sampling regime has been used in attempt to gain a consistent mean concentration value.

This experiment represents a snapshot after 2 full years of growth. Other physiological factors such as drought or disease may also affect the relationship between leaf and stem metal concentrations. It is encouraging that much of the variation shown in figures 5.4.1 is low and can be explained within the 95% confidence intervals shown. In this case it may be more realistic to use the confidence intervals to predict a possible concentration range from one parameter rather then using an individual figure.

More data from field trials are needed to prove or disprove if these correlations are valid in a long-term field situation.

5.5 Relationships in metal distribution II: Soil and above ground biomass

Another key issue when investigating generalized relationships is the soil metal concentration and its relationship with uptake into the above-ground biomass of the plant. If such relationships exist it may be possible to predict how much metal could be removed from the soil at a specific concentration. There are several methods of measuring and extracting metals from soil (Chapter 1, Section 5.1), and current data may reveal which is the most effective predictor of metal accumulation in above-ground tissues. Only stem data was assessed as a measure of above ground metal uptake, as this may prove to be a longer term metal sink than foliage, and is more difficult to measure non-destructively.

Initial relationships between stem uptake and soil concentration were conducted using Pearsons product moment correlations (Table 5.5.1) using transformed data (log x+1) for stem (Year 3) and corresponding soil concentrations for EDTA extractable and Total soil values in year 1, for each species, pooled over all sites and for each metal. Only significant results are shown ($p<0.05$). Statistically significant results were subjected to additional scrutiny, by calculation of r^2 values as shown in table 5.5.2. and scatterplots in Figures 5.5.1 to 5.5.3.

Table 5.5.1. Pearson's correlation matrix for EDTA extractable and total (HNO_3 extractable) soil concentrations against stem concentrations in Year 3 for Cd and Zn. Values shown are person product moment correlation (r) and p values

	ALN	BET	CAL	CLS	GER	GHY	LAR	ORM	TOR	TRI	All willow	All poplar
Cd EDTA			.976 <0.001 **	.891 0.017*	.885 0.002**	.792 0.060		Nt	.958 0.010*	.791 0.034*	.832 <0.001 **	.789 <0.001 **
Cd Total			.976 0.004**	.988 0.012*	.706 0.183	.889 0.111		Nt	.831 0.169	.965 0.008**	.712 <0.001 **	.869 0.011*
Zn EDTA				.842 0.035*	.824 .0006**			Nt				
Zn Total					.708 0.033*			Nt				

Table 5.5.2. r^2 values for significant correlations produced in Table 5.5.1 showing EDTA extractable and total (HNO_3 extractable) soil concentrations against stem concentrations in Year 3 for Cd and Zn. Bold figures boxes show highest value.

	ALN	BET	CAL	CLS	GER	GHY	LAR	ORM	TOR	TRI
Cd EDTA	0.1		**0.99**	0.88	**0.95**	0.78		Nt	**0.97**	0.85
Cd Total			0.84	**0.95**	0.83	**0.87**		.nt	0.89	0.88
Zn EDTA				0.73	0.74			Nt		
Zn Total								nt		

The r^2 values indicate strong correlations between EDTA and total soil concentrations and stem tissue for Cadmium in all *Salix* and *Populus* taxa. ORM was not tested due to a lack of data. Overall concentrations of Cd for *Populus* taxa are generally lower than those for *Salix*. Of the 4 Salix taxa, CAL GER and TOR all have significantly higher r^2 values with EDTA than with total soil. The exception is CLS where the r^2 total soil value is marginally higher.

Figure 5.5.1. Concentration relationships for tree species showing a significant relationship between stem Cd (Year 3) and total soil Cd (Year 1). Lines either side of indicated extrapolated correlation line are 95% limits.

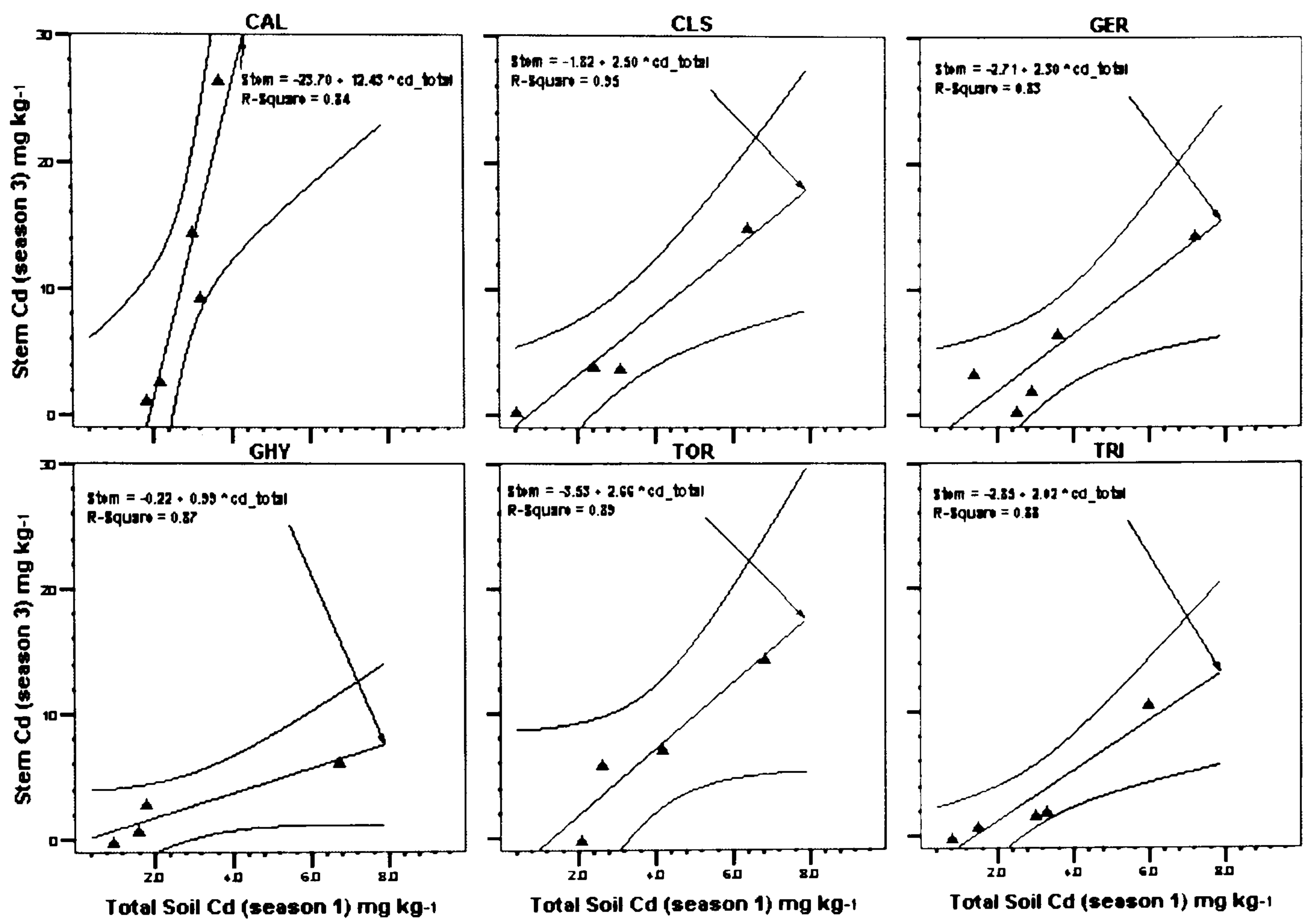


Figure 5.5.2. Concentration relationships for tree species showing a significant relationship between stem Cd (Year 3) and EDTA extractable soil Cd (Year 1). Lines either side of indicated extrapolated correlation line are 95% limits.

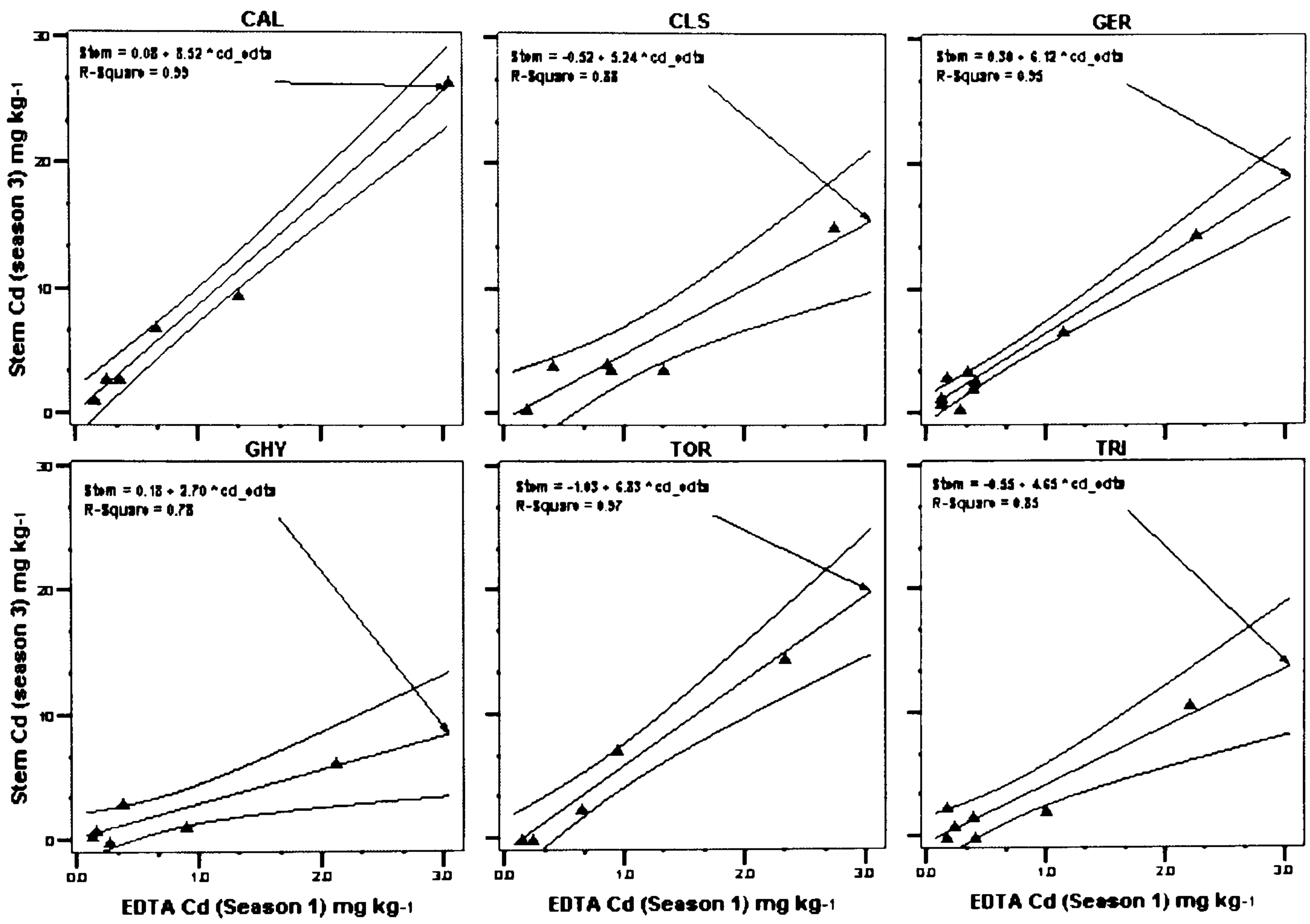
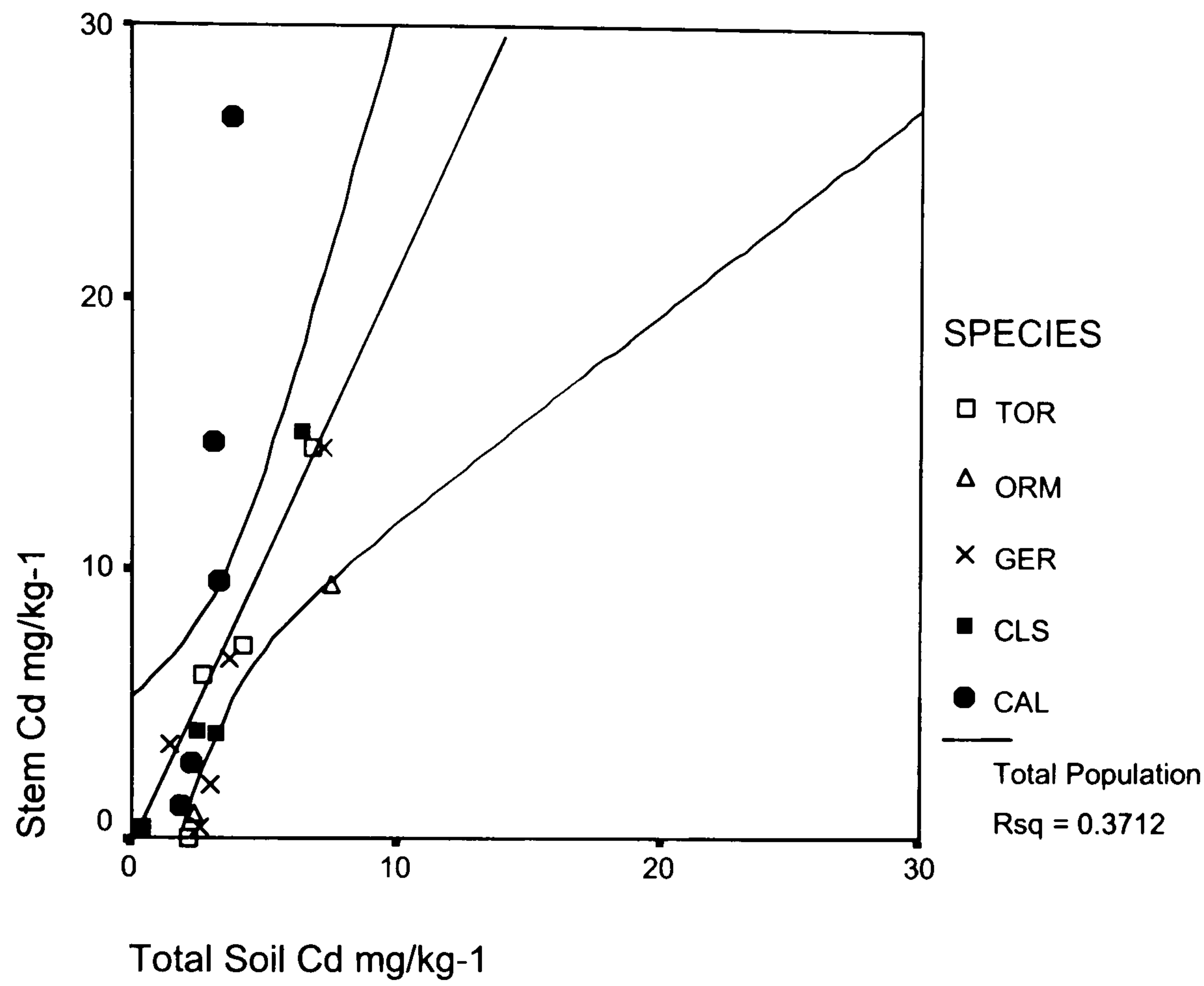
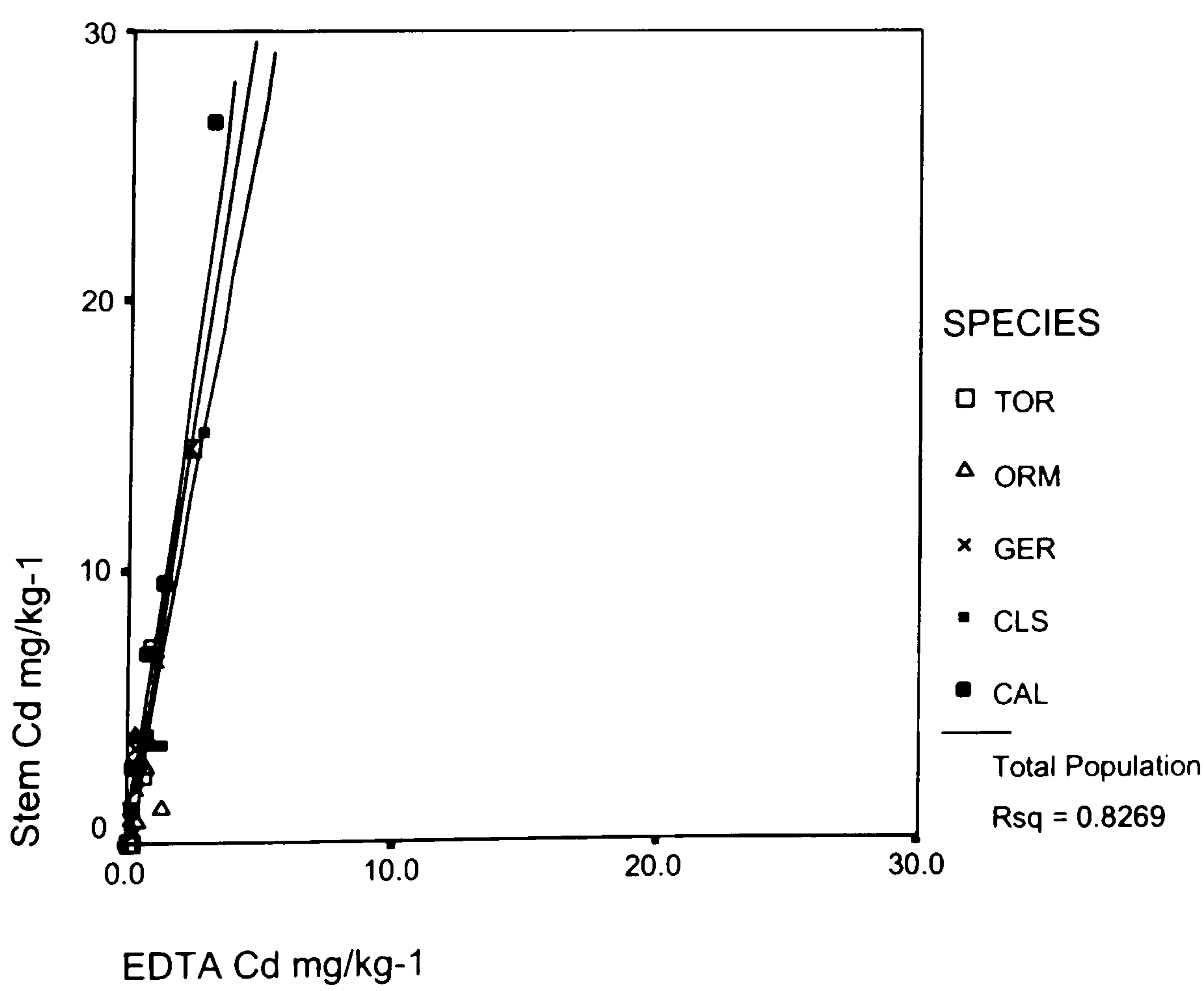


Figure 5.5.3. Relationship between Cd concentrations in stems of all Salix *taxa and (i) total soil Cd (upper graph) and (ii) EDTA-extractable Cd (lower graph)*





Soil metal and stem metal relationships - Overview

Although statistically it appears that total soil Cd and EDTA-extractable Cd are good predictors of metal concentrations in above-ground biomass, it is not until the entire genus is considered together that it becomes evident EDTA is a significantly better indicator. Soil concentrations of Cd are generally low in the present study and additional data with high concentration values would give extra confidence to the predictions. However, the present study examined real Brownfield situations where low level Cd contamination (as opposed to high level contamination) occurs on many sites. With different extracting solutions Cd in above ground biomass is strongly correlated to soil concentration, which has also been reported elsewhere (Vandecasteele *et al.* 2002).

EDTA would appear to be a better predictor of stem uptake, especially when considering *Salix* as a whole genus (Fig 5.5.3). Total soil concentrations may be useful in certain species. Zn does not exhibit the same range of correlations across all taxa, although some individual taxa show strong relationships with EDTA extractable concentrations of Zn. Additional longer term field data is really required to verify these results.

$CaCl_2$ may also be a useful predictor of stem concentrations, however, recoveries were in many cases were below detection limits, not allowing a proper statistical evaluation (see section 5.1).

5.6 Changes in seasonal metal distribution

Studies that have previously examined the use of *Salix* for phytoremediation have noted seasonal or annual differences between metal uptake in the leaves. Changes within a growing season occur, for example, due to elevated metal concentrations in leaves prior to senescence. Using data from field trials over 3 seasons, changes in metal content from one growing season to the next can be examined. If changes exist and are substantial, predictions of foliar uptake may be difficult, and may impact on optimal time of harvest.

Data for foliar metal concentrations at the end of growth season 2 (1^{st} full growing season) and growth season 3 were compared for each metal and each species (pooled across all sites) using a paired t-test on transformed data (log x+1) to ascertain if there were any significant differences. The results can be summerised as follows:

- **Cadmium**: No significant differences between year 2 and year 3 for all species / taxa
- **Copper**: ALN significantly different year 2 to year 3 $p=0.004$ ($p < 0.01$)
- **Nickel**: GHY ($p=0.03$) significantly different year 1 to year 2 ($P < 0.05$)
- **Lead**: ALL species except LAR and GHY significantly different ($p<0.01$)
- **Zinc**: TRI ($p=0.026$) significantly different year 1 to year 2 ($P < 0.05$)

Overall there is little difference between the quantities of metals taken up by foliage from Years 2 to 3, with a few exceptions. The result for Pb could be interpreted as misleading, concentrations are all extremely low and close to detection limits of the analytical techniques used. Small deviations that are most likely analytical error may be manifested as significant differences. The mean foliar concentration of Pb in Year 2 is 1 mg kg^{-1} and the maximum in Year 3 is 5 mg kg^{-1} across all sites and species, representing minimal change.

ALN is able to accumulate significant concentrations of Cu in comparison to other species (Fig 5.2.2) with the highest mean foliar uptake of all species and a low variance, these results imply more Cu was accumulated in the foliage in Year 3. TRI is able to accumulate large amounts of Zn in foliage, however, the leaves of TRI have the largest area of any species used in the experiment. It is therefore difficult to achieve a homogenous sample which may contribute to apparent seasonal differences. Additional data collection should emphasize sampling and processing leaves of a known area to ensure parity between seasons and test if leaf area is a factor. Alternatively it may be another accumulation mechanism.

It is unknown if the relationship between Year 2 and Year 3 foliar levels would change in the future as fluxes of stem and soil metals may change. However, in the context of this study it is an important factor as data from foliar in years 2 or 3 can be used as a comparison in assessments of uptake. Further research is required on ALN (copper) and TRI (zinc) to see if differences in foliar concentrations are due to an underlying physiological function or merely insufficient sampling and analysis practice.

5.7 Changes in soil metal concentration after 3 years of tree growth

Previous sections in this chapter have established that some tree species or taxa are able to accumulate metals in their above ground tissues. Consideration should be given to the potential for concentrations of metals present in soil to change after 3 years of tree growth. Processes will occur within the soil and root interface to allow metal uptake into above ground tissues and tree root systems are know to affect soil metal availability (Chapter 1). This comparison is has been assessed using EDTA extractable metal concentrations as they may represent a more realistic measure than total metal concentrations, but at a quantifiable limit of detection, unlike $CaCl_2$ where recovered concentrations were to low to allow statistical analysis.

Figure 5.7.1 presents a visual indication of the changes in EDTA soil extractable metals from Year 1 to Year 3. It cannot, however, accurately reflect the site changes as it shows mean pooled data for all species, not allowing for variation that may be present within each plot. A paired t-test will produce a more accurate statistical result comparing samples directly from Year 1 to Year 3.

All analysis was conducted using transformed (log x+1) data. Figures in italics were not significant, but close to the significance level of $p<0.05$ and may warrant some consideration. KS tests for normality were conducted and all interactions with the exception of Cd on MER 3 were found to be normally distributed. Due to the presence of non-normal data, the comparison was also conducted using a non parametric equivalent of the paired t test, the wilcoxian signed ranks test. Results were comparable to those achieved with a paired t test and therefore only the paired t-test data is presented in tables 5.7.1 and 5.7.2.

Figure 5.7.1. Mean concentrations of soil EDTA extractable metals in year 1 and in year 3 for all experimental plots

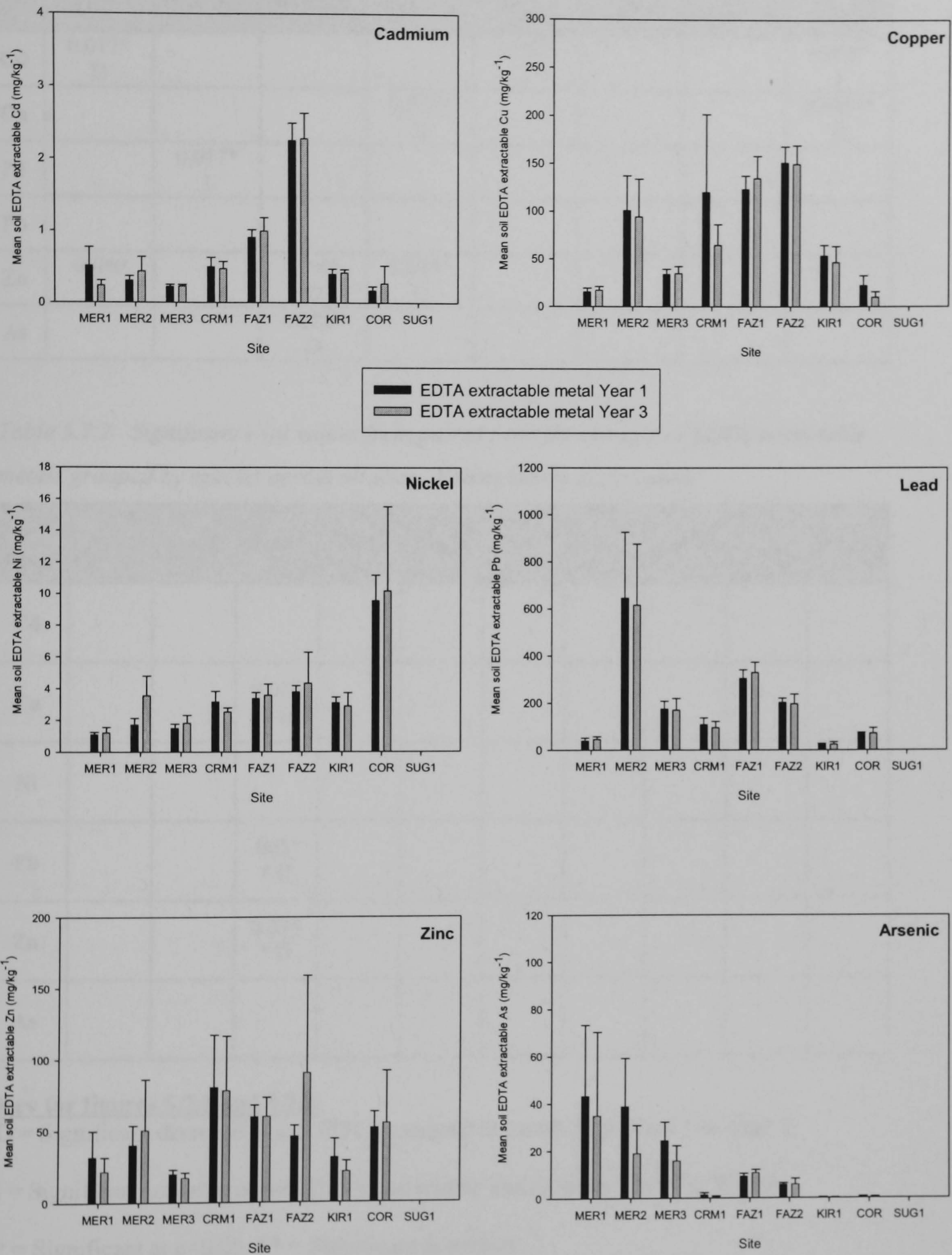


Table 5.7.1. Significance (p) values from paired t-test for changes in EDTA extractable metals from Year 1 to Year 3 on each site. Values shown are p values.

	MER 1	MER 2	MER 3	CRM 1	KIR 1	FAZ 1	FAZ 2	COR
Cd	0.017* D				0.033* I			0.028* I
Cu				0.038* D				0.002** D
Ni		0.017* I						
Pb								
Zn	*0.060 D*		*0.065 D*	0.023* D				
As			0.021* D					

Table 5.7.2. Significance (p) values from paired t-test for changes in EDTA extractable metals grouped by species across all sites. Values shown are p values

	ALN	BET	CAL	CLS	GER	GHY	LAR	ORM	TOR	TRI
Cd										
Cu			0.038 * D							
Ni										
Pb			.005* * D							
Zn			0.035 * D							
As										

Key for figures 5.7.1 and 5.7.2
D = Significant decrease in soil EDTA extractable metals from Year 1 to Year 3.

I = Significant increase in soil EDTA extractable metals from Year 1 to Year 3.

* = Significant at p<0.05 ** = Significant at p<0.01

Table 5.7.1 presents a range of results, not immediately showing any consistent pattern. The changes in Cd concentration, although statistically significant, showing an increase in KIR and COR and a decrease on MER 2 represent extremely small changes in actual field Cd concentration; mean concentrations for all 3 sites are less than 0.5mg kg^{-1} EDTA extractable Cd. A similar pattern of low recoveries is repeated with Ni EDTA concentrations.

Copper, Zinc and Arsenic are present and detection limits for these elements are not an issue. Copper concentrations have been significantly reduced at CRM1 and COR, and Zn concentrations have decreased at CRM 1. Although not at a significant level ($p<0.05$) MER 1 and MER 3 display p values marginally outside significant ranges suggesting some possible decrease in soil Zn. Arsenic also showed a significant decrease on MER 2.

The same test was conducted by examining the same species or taxa across all sites (Table 5.7.2). Although comparisons of year 1 and year 3 EDTA soil concentrations immediately below all species were tested the only significant results occurred for CAL, which significantly reduced Cu, Pb and Zn EDTA concentrations from year 1 to year 3.

Overview

Increases of Cd and Ni in soil during the experimental period indicated on Table 5.7.1, whilst statistically significant, do not necessarily translate to real field significance. If they are correct and not an error due to low analytical recovery values they would represent an actual increase of only between 0.1 – 0.25 mg kg^{-1} EDTA extractable metal in soil.

Only one tree species, CAL, showed a significant decrease of metals (Zn, Pb and Cu) in samples taken from soil below these plants. Although CAL does accumulate these metals (Fig 5.2.1) other *Salix* taxa accumulate similar above ground concentrations with no discernable change in soil EDTA metal concentrations. It may that another uptake mechanism is influencing metals around CAL, such as increased root uptake or rhizosphere processes that were not quantified and would benefit from further investigation.

Although previous studies in this chapter have reported elevated uptake of Zn and Cu in tree species (Section 5.2) it is not possible to conclusively state that the reductions in soil metals were due to tree performance. Other site processes such as changes in oxidation state, pH or leaching may affect metal balances in a more profound way. The ability for tree root systems to influence soil metal availability is discussed in Chapter 1.
Other studies of changes in soil metals under *Salix* planting provide mixed results, Pulford *et al.* (2002) reported a rise in EDTA extractable levels of Cd, Cu, Ni and Zn on areas planted with the *Salix* taxa 'Rosewarne White' when compared to unplanted areas. However, Eriksson and Ledin (1999) reported a 30 – 40% drop in $CaCl_2$ and exchangeable Cd in 10 year old *Salix* stands compared to unplanted areas.

Many factors contribute to metal availability on a site specific level and to form more accurate conclusions longer term assessments are necessary to accurately study change in mobility. This is a frequently overlooked element of phytoremediation or restoration schemes. Data suggests that trees can grow successfully on contaminated land, but very rarely are the long term effects on metal speciation considered, this is critical in assessment of ecotoxicity and human health. The limited investigations conducted in this experiment suggest that mobilization of metals is not significantly increased after 3 years of growth, which strengthens an argument that tree planting provides a degree of phytostabilisation as well as a landscape improvement and plant uptake.

Chapter 6: Survival, Growth and Yield

6.1 Introduction

The planting scheme used in this study is applicable to phytoremediation, community forestry and biomass crop production. All require successful establishment and continued survival and growth of the plants. This chapter considers the relative success of establishing SRC (or fast-growing trees) on contaminated sites, examining growth, mortality and yield over three growing seasons. Environmental factors that impact on the success of tree establishment and their possible effects at the experimental sites are also considered.

The success of phytoremediation will be related to the proportion of planted trees that survive on the site, somewhat irrespective of their potential capacity for uptake or stabilisation. The amount of biomass produced may also be critical if the remediation mechanism is based upon phytoextraction (Chaney *et al.* 2000; Pulford and Watson 2003). For community forestry, excessive tree mortality will reinforce negative effects on land or in areas that already have a cycle of deprivation (Perry and Handley 2000). Replacing failed trees may be impractical and prohibitively expensive for community led schemes. If the trees grown are to be used for timber related products or other commercial concern then achieving the best yield possible will be important to maximise revenue (DEFRA 2002a). Continued high yields may also be important to sustain any commercial enterprise and therefore allow effective ongoing management (Mitchell *et al.* 1999).

6.2 Monitoring and measurement of biomass

An annual census was conducted for each plot to record the presence or absence of individuals of each species or taxa, plus a measure of overall height. In the case of multi-stemmed trees, the height of the tallest stem was recorded. Because of the large number of plots and trees not every stem was measured; those on the edges of species blocks were excluded to remove potential error due to edge effects. This allowed measurement of approximately 70% of the plot in order to provide an accurate representation of growth and mortality. Full details of monitoring techniques are provided in Chapter 3.

Destructive measurements to assess total yield or biomass were conducted at the end of Season 3 for *Salix*, *Populus* and *Alnus* species (see Chapter 3). Data presented below for survival and growth excludes the small plot at Corus (COR1) site. This is due to the large differences in experimental design between COR 1 and all other plots in the study, and because too few trees were planted to make accurate assessments of mortality and biomass. The site SUG 1 is excluded from several of the comparisons in this section due to different coppicing regimes. It is, however, included in a separate section of this chapter.

6.3 Results of Establishment and Mortality

Figure 6.1 shows the **cumulative** increase in mortality of species at each site over 3 growing seasons. Mortality and establishment were considered in several different ways:

6.3.1 Establishment and mortality

The establishment of plants by the end of the first full growing season is critical as it is the time when stock may be lost and is most susceptible, especially for bare rooted coppice species or taxa and those planted on marginal or problematic land (Bending and Moffat 1999; Putwain *et al.* 2003). If mortality is high at an early stage, cost implications to replace failed stock will be great and potentially prohibitive for the scheme to continue. Values for 2001 percentage mortality (Figure 6.1) were used to assess the relative success or failure of establishment of each species or taxa at each site. With the exception of larch, which consistently had high levels of mortality on all sites, establishment in Year 1 was good. Sites range from almost zero percent mortality (for Faz 1, Mer 3), but did not exceed 20% for any other site and were generally much lower. An exception was on Merton 1 where Year 1 mortalities typically lie in the range from 20% to 50%. Some of this loss can be attributed to vandalism in Year 1, as this was the only plot to suffer significant removal and destruction of stools that were not replaced. It is also the only plot to be situated on a steep incline, which may have led to problems of moisture retention, exposure and soil stability. Sugar Brook (SUG 2) had a elevated mortality rate in Year 1, probably attributable to weed control issues during the season after cutback and the removal of an entire row through incorrect weed management and strimming.

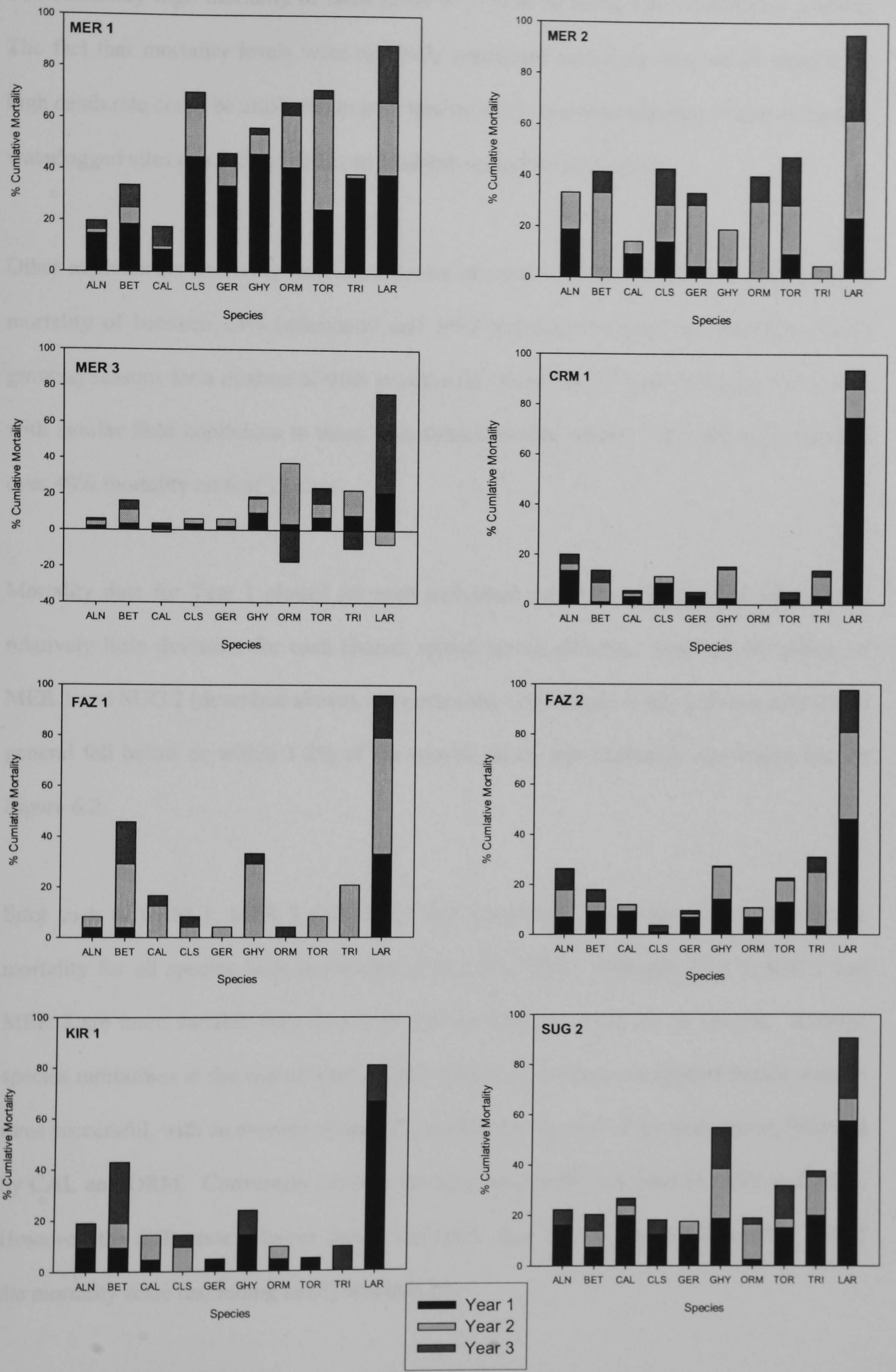


Figure 6.1. Cumulative mortality for all species on each plot growing over 3 seasons.

The uniformly high mortality of larch (20% to 77% at all sites) was a significant problem. The fact that mortality levels were relatively consistent across all sites would suggest the high death rate could be attributed to poor quality stock, incorrect planting season or method, waterlogged sites or a lack of ability to establish on low fertility sites.

Other experiments involving the establishment of trees on derelict land have cited levels of mortality of between 23% (minimum) and 60% (maximum) cumulative mortality over 3 growing seasons for a mixture of trees growing on closed landfill sites (Putwain *et al.* 2003), with similar field conditions to those investigated in this project. The same study showed over 40% mortality on 6 of 11 sites.

Mortality data for Year 1 plotted for each individual species or taxa (Figure 6.2) showed relatively little deviation for each species spread across all sites. With the exceptions of MER 1 and SUG 2 (described above), all species showed little deviation between sites and in general fell below or within 1-2% of the species mean, represented by the dashed line on Figure 6.2.

Sites such as CRM 1, MER 3 and FAZ 1 fell consistently below the average percentage mortality for all species, with mortalities of less than 10%. Although FAZ 2, KIR 1 and MER 2 are more variable they do not deviate far from the mean for all species. Average species mortalities at the end of Year 1 were similar, if ranking was applied *Betula* was the most successful, with an average of only 7% mortality at the end of the first season, followed by CAL and ORM. Conversely *Larix* is the least successful, followed by GHY and ALN. However, the difference between *Betula* and GHY, that can be considered at either end of the mortality scale (excluding larch) was only 6%.

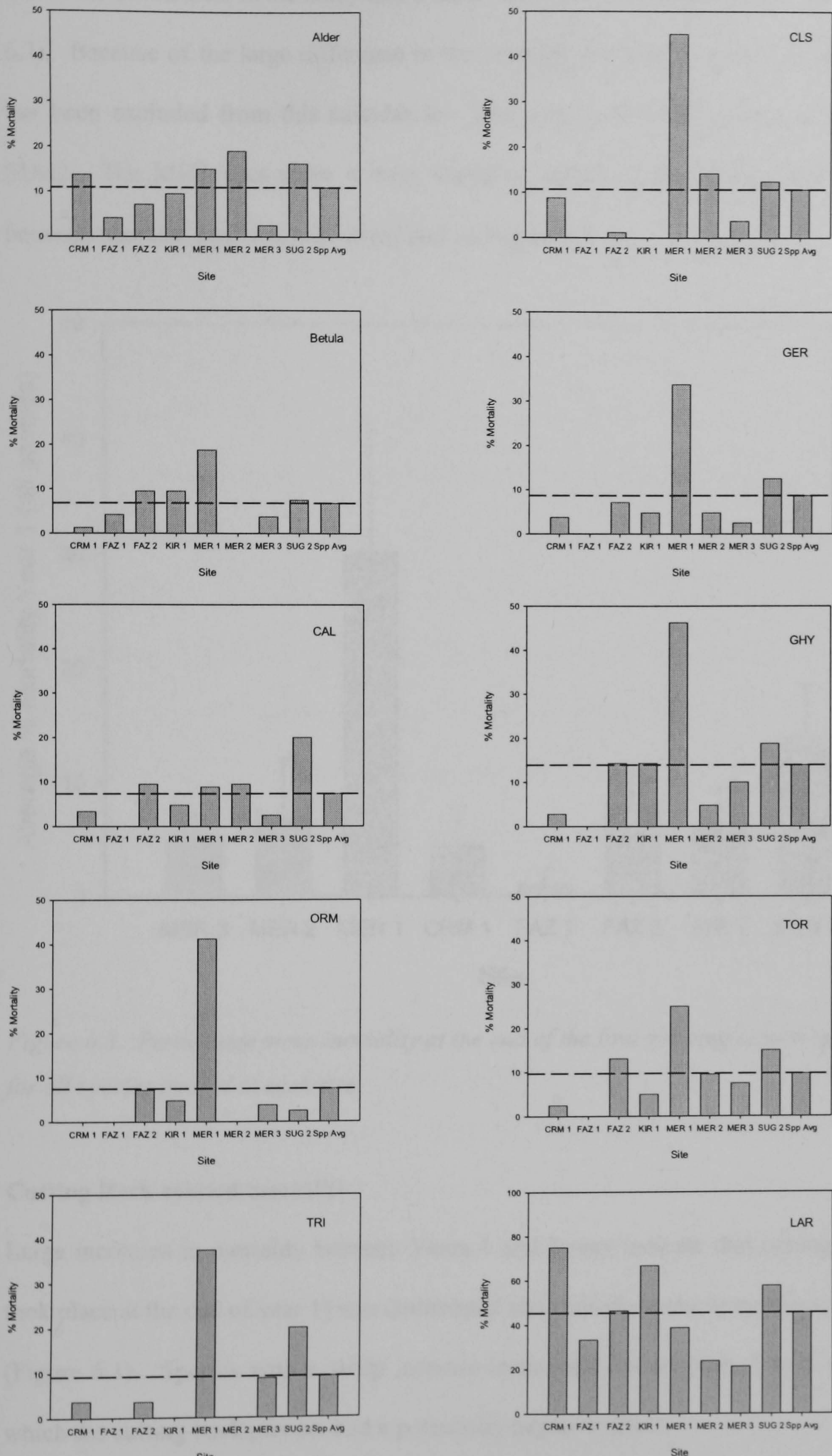


Figure 6.2. Percentage mortality at the end of the first growing season (pre cut-back) for individual species on each plot. The dashed line represents average mortality for those taxa across all sites.

Six of the seven sites in the study had a mean total year 1 mortality of less that 10% (Figure 6.3). Because of the large difference in the mortality of larch compared to other species it has been excluded from this calculation. The poorest performing sites were MER1 and SUG2. The MER sites show a large variation, reflecting the difference in performance between taxa that can clearly be identified on Figure 6.3.

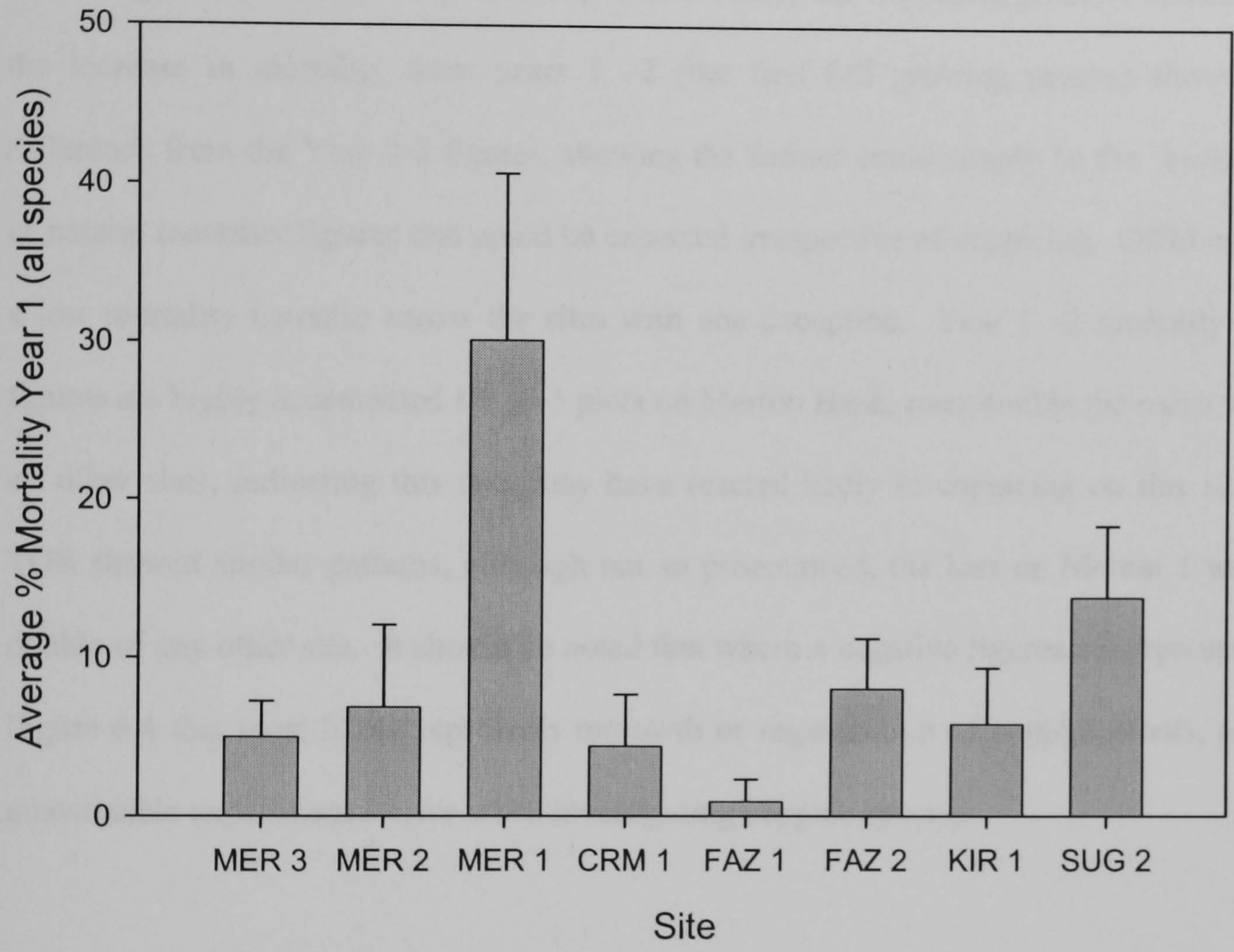


Figure 6.3. Percentage mean mortality at the end of the first growing season (pre cut-back) for all species pooled at each site

Cutting Back related mortality

Large increases in mortality between Years 1 and 2 may indicate that cutting back (which took place at the end of year 1) was detrimental and caused excessive numbers of trees to die (Figure 6.1). Species with a sharp increase in mortality from years 1 to 2 were those in which the cutting-back process had a potentially negative effect.

An easier way to examine these data is to focus on the changes in mortality from Season 1 to Season 2 in isolation and to compare them with the changes in mortality from seasons 2 to 3.

This gives an indication of the potential level of mortality if the trees had not been coppiced (Figure 6.4). *Alnus*, *Betula* and *Larix* were not cutback at the end of the first growing season and these species are discussed separately later in this section.

Figure 6.4 shows CAL, CLS and GER had small increases in mortality from Year 1 to 2, indicating that these taxa were relatively unaffected by the coppicing process; in many cases the increase in mortality from years 1 –2 (the first full growing season) showed little difference from the Year 2-3 figures, showing the former could simply be the 'background' or natural mortality figures that could be expected irrespective of coppicing. ORM exhibited a low mortality increase across the sites with one exception. Year 1 –2 mortality change figures are highly accentuated for all 3 plots on Merton Bank, over double the mean value of all other sites, indicating this taxa may have reacted badly to coppicing on this site only. TOR showed similar patterns, although not so pronounced, the loss on Merton 1 was over double of any other site. It should be noted that where a negative figures are represented on Figure 6.4 this most likely represents regrowth or regeneration of coppice stools, and is a unavoidable experimental error when investigating coppice species.

The most notable observation was the consistently high increase in mortality from Years 1 to 2 on all sites for GHY and TRI, the only two poplar species. These are especially noticeable as large increases in mortality for the year after cutback (1 – 2) occurred on sites such as CRM 1, FAZ 1 and FAZ 2 and MER 3, all of which exhibit low levels of mortality for all other species. The supposition that they reacted badly to being cut back after Year 1 is also manifest in other ways. The increase in mortality for Year 1 to 2 is increased rapidly (Figure 6.4), but in <u>all</u> sites the increase from Years 2 to 3 is lower, in many cases zero, indicating those species that survived the cutting back thrived the following season. Establishment of both these species was generally low on all but a few sites (Figure 6.1), the better establishment in the Year 1 to 2 season.

It is uncertain why poplars reacted negatively to the cut back, although this has been noted in other studies (Herve and Ceulemans 1996), specifically with reference to poor competition at regrowth, causing the loss of smaller shoots. Another possible suggestion is the poor performance of *Populus* generally at more northern latitudes when compared to *Salix*. In addition poplars have a high degree of apical dominance (Tabbush and Beaton 1998; Laureysens *et al.* 2003) and seldom produce more than 2 or 3 stems after cutback. If willows respond with faster growth rates after cutback it may be that poplars were out-competed in the mixed stands. Higher mortalities post cutback have been reported in other studies, with a possible explanation being the poor rooting capacity of *P. deltoides* on soils with higher bulk densities. Better cropping, certainly in the establishment season can also be achieved by a single 4-year harvest as opposed to two 2 year harvests (Armstrong *et al.* 1999). This should be noted for future harvesting and yield research on brownfield land.

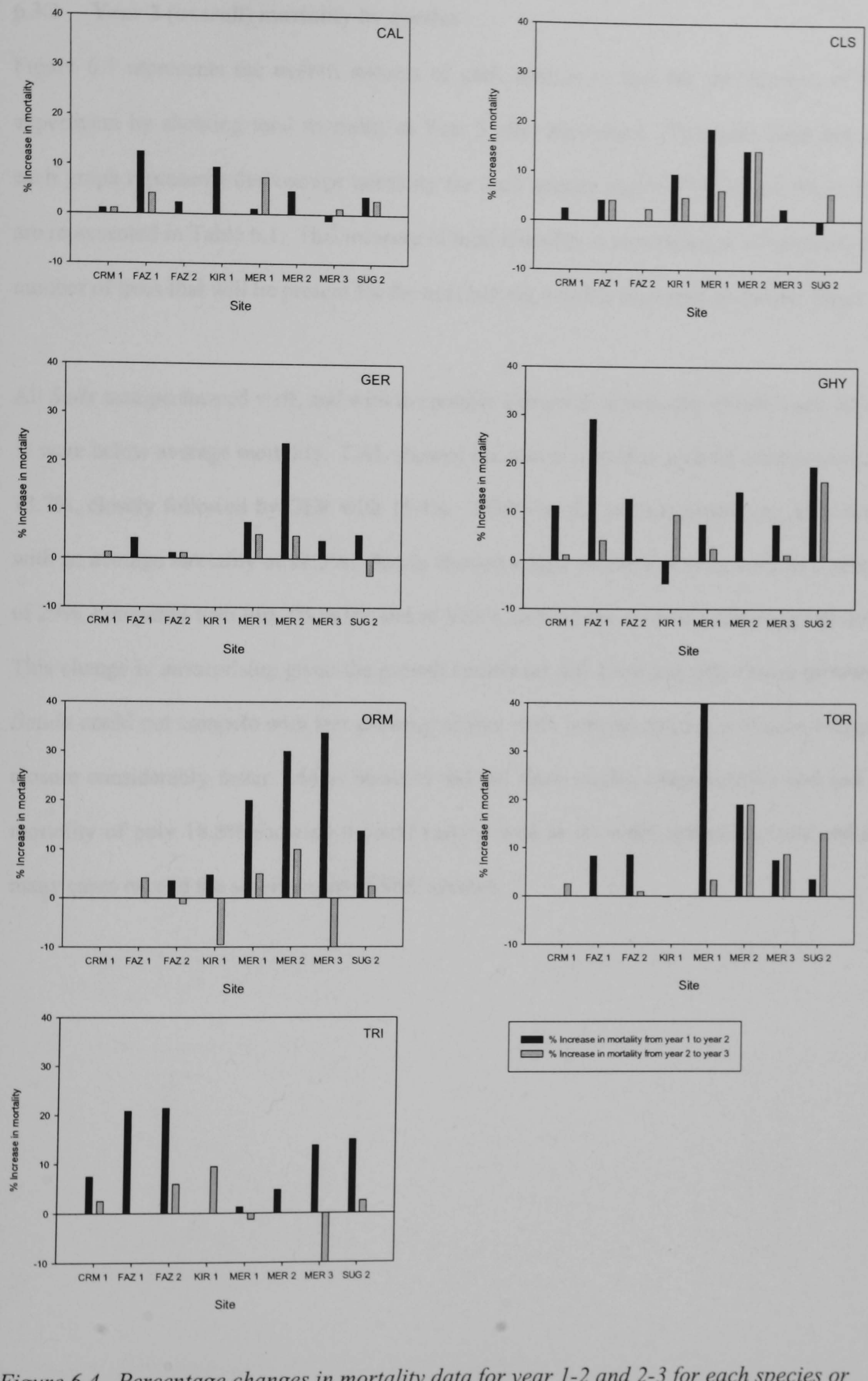


Figure 6.4. Percentage changes in mortality data for year 1-2 and 2-3 for each species or taxa on all sites.

6.3.2 Year 3 (overall) mortality by species

Figure 6.5 represents the overall success of each species or taxa for the duration of the experiment by showing total mortality at Year 3 after harvesting. The solid black line on each graph represents the average mortality for each species across all sites and these data are represented in Table 6.1. This measure of total mortality is significant as it represents the number of trees that will be present for the next harvest rotation assuming no further losses.

All *Salix* taxa performed well, and with the notable exception of two sites (MER 2 and MER 1) were below average mortality. CAL showed the lowest mortality with an average of only 13.7%, closely followed by GER with 15.4%. TOR was the poorest performing *Salix* taxa with an average mortality of 26.3%. *Betula* showed a high overall mortality with an average of 29%, compared with just 7% at the end of Year1, at this time the best performing species. This change is unsurprising given the growth conditions and close spacing, slower growing *Betula* could not compete with fast growing willow, with coppice species achieving canopy closure considerably faster. *Alnus* however did not show similar characteristics and had a mortality of only 18.8% showing it could survive well in the multi-species mixture and in many cases exceed the survivorship of SRC species.

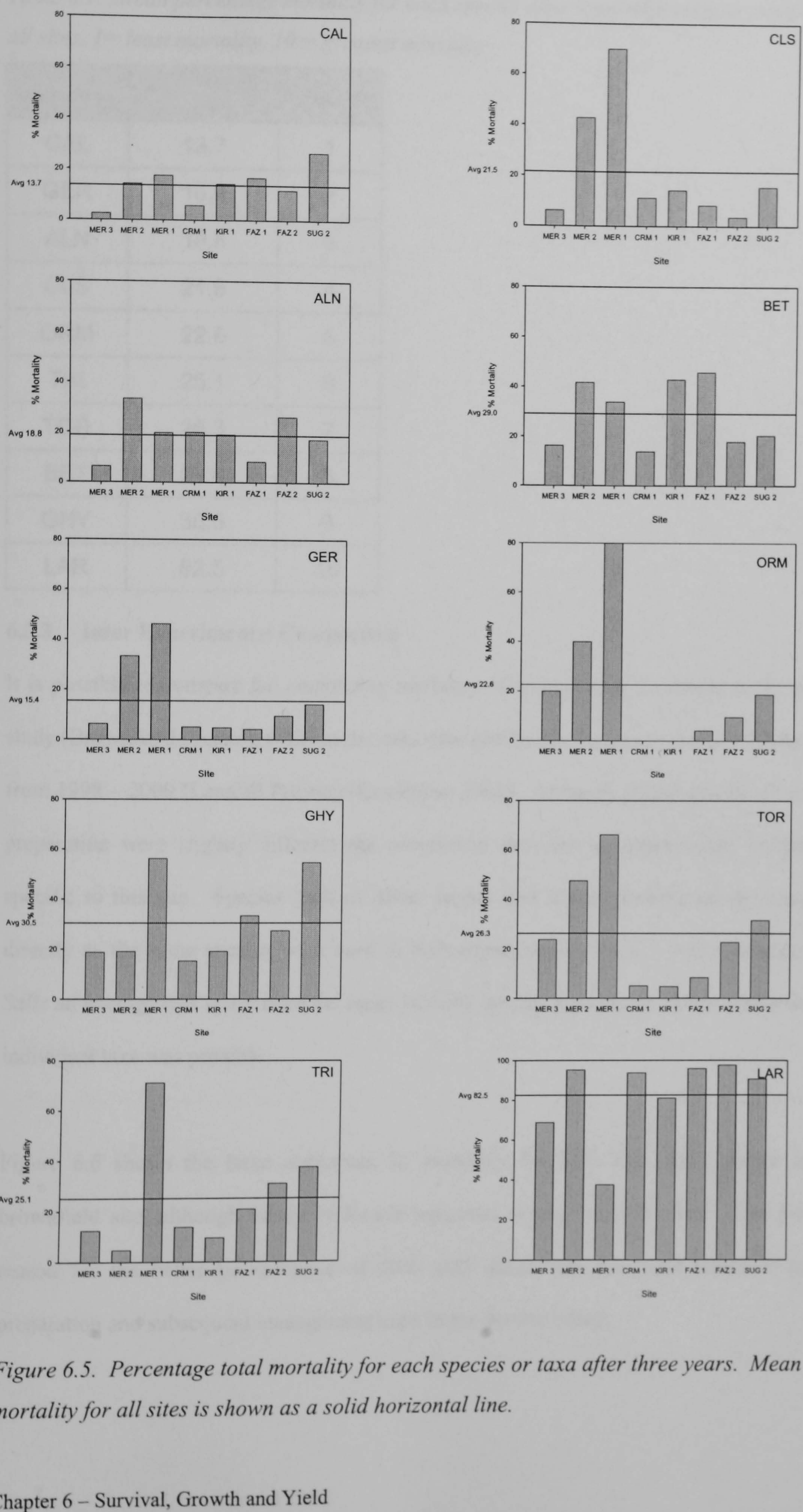


Figure 6.5. Percentage total mortality for each species or taxa after three years. Mean mortality for all sites is shown as a solid horizontal line.

Table 6.1. Mean percentage mortality for each species after 3 growing seasons pooled over all sites. 1= least mortality, 10 = greatest mortality

Species	% Mean Mortality	Rank
CAL	13.7	1
GER	15.4	2
ALN	18.8	3
CLS	21.5	4
ORM	22.6	5
TRI	25.1	6
TOR	26.3	7
BET	29.0	8
GHY	30.5	9
LAR	82.5	10

6.3.3 Inter Experimental Comparison

It is possible to compare the cumulative mortality of species after 3 seasons in the present study (Brownfield Project), with similar data obtained from a field experiment at Kirby Moss from 1998 – 2000 (Landfill Project) (Rawlinson 2001). Although planting methods and site preparation were slightly different the experiment provides an approximate comparison, specific to this site. Species such as *Alnus incana* and *Betula pendula* can be compared directly as the same species were used in both experiments over a 3 year timescale. For *Salix* an average was taken from the range of *Salix* species used, as no direct comparison for individual taxa was possible.

Figure 6.6 shows the large reductions in mortality for *Salix* and *Alnus* grown on the brownfield site, although losses of *Betula* remained similar on both plots. One possible reason for the better performance of *Salix* and *Alnus* was the more intensive ground preparation and subsequent management used in the present study.

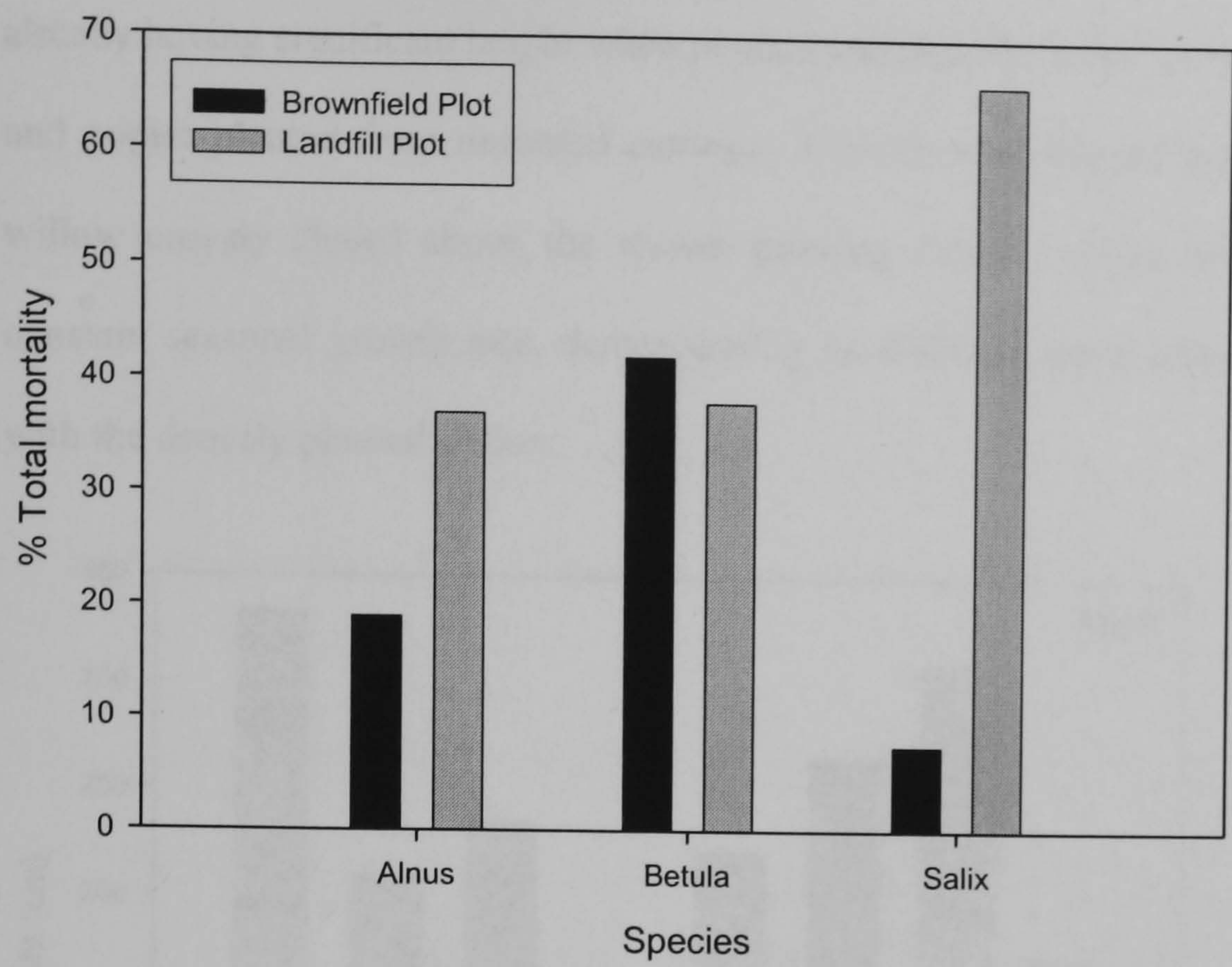


Figure 6.6. Percentage mortality at Year 3 for similar species from two different experiments (Brownfield project and Landfill project) at Kirby Moss.

6.4 Tree growth

Height data were collected from all species over 3 growing seasons. This provided a simple and effective growth measurement for broadleaved species such as *Betula, Alnus* and *Larix* and can be used to assess annual increment growth. The usefulness of this approach is limited in coppiced taxa such as *Salix* and *Populus* that produce multiple stems from each stool. Considering height only and not stem number or diameter would not be an accurate reflection of growth or biomass production. In addition this method is prone to error with species that have been cut back, for example, after the first year of growth and then failed to re-grow to the initial height, effectively producing negative growth increments. Data for height increment growth of *Alnus* and *Betula* are shown in Figure 6.7; due to experimental error there were no height increment data for Year 3 on KIR1 and SUG 2. *Larix* has been omitted as, due to the extremely high mortality rates, there were insufficient data for each site. Alder was also assessed using the biomass technique described later in this chapter.

Growth of *Betula* was superior on many sites in the first year, this was due to; (a) stock already having significant height when planted and (b) little initial competition due to willow and poplar planted from unrooted cuttings. Growth was reduced season by season as the willow canopy closed above the slower growing *Betula*. *Alnus* by contrast exhibited a constant seasonal growth rate, demonstrating its ability to grow effectively in competition with the densely planted willow.

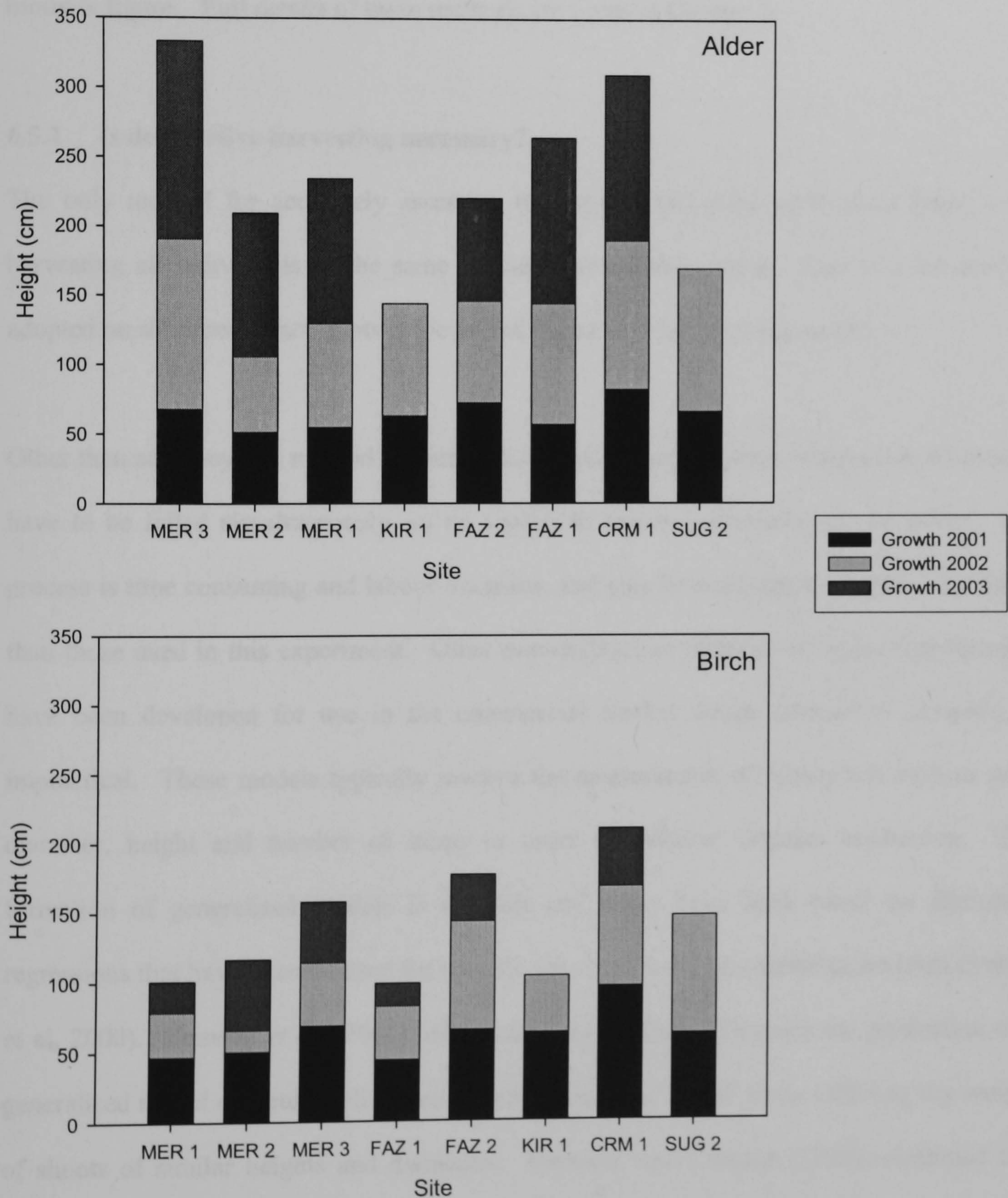


Figure 6.7. Height increment growth for Alder and Birch over 3 growing seasons, 2000 – 2003.

6.5 Biomass Assessment as a measure of growth.

Measurement of the growth and production of coppice species is generally determined by total biomass, the weight of all stems of the tree, corrected for moisture content and expressed as a 'weight per unit area', typically in oven dry tonnes per hectare, (odt ha^{-1}). This figure accounts for height, girth, taper and most importantly number of stems produced by individual plants. It will also take into account losses though mortality on the overall net biomass figure. Full details of these methods are given in Chapter 3.

6.5.1 Is destructive harvesting necessary?

The only method for accurately assessing the growth and yield of biomass trees is via harvesting all individuals of the same species and weighing them. This was the method adopted on all experimental plots at the end of the second full growing season.

Other than accuracy this method has many drawbacks. For a realistic comparison all species have to be felled simultaneously, so no season to season assessment can be made. The process is time consuming and labour intensive, and may be impractical for plots any larger than those used in this experiment. Other non-destructive methods of biomass assessment have been developed for use in the commercial market where destructive sampling is impractical. These models typically involve the measurement of parameters such as stem diameter, height and number of stems in order to estimate biomass production. The formation of generalised models is difficult and many have been based on allometric regressions that have been devised for specific site / species / age combinations (Ben Brahim et al. 2000). Heinsoo *et al.* (2002) identified several factors that make the production of a generalised model difficult such as soil nutrient status and age of stems affecting dry weight of shoots of similar heights and diameters. Verwijst and Telenius (1999) reinforced that weight diameter relationships are specific for age, species and stands and that a biomass estimation procedure is ultimately a compromise.

Current models being developed in the UK for willow and poplar species involve the long term monitoring of over 40 sites in the UK and Ireland (Armstrong 1997; Tubby and Armstrong 2002).

6.5.2 Field Biomass Assessment

Biomass was assessed via destructive harvesting during November and December 2002, the end of the 3rd growing season, representing 2 full growing seasons since the trees were cutback at the end of year 1. Biomass assessments were made of all *Salix* and *Populus* taxa, plus *Alnus*, which has the potential to be used as a short rotation crop (Johansson 2000; Uri *et al.* 2002). Annualised biomass production of all species for each plot in odt ha^{-1} yr^{-1} using data from 2 full growing seasons (Years 2 and 3) is shown in Figure 6.8, with means of the data summarised in Figure 6.9.

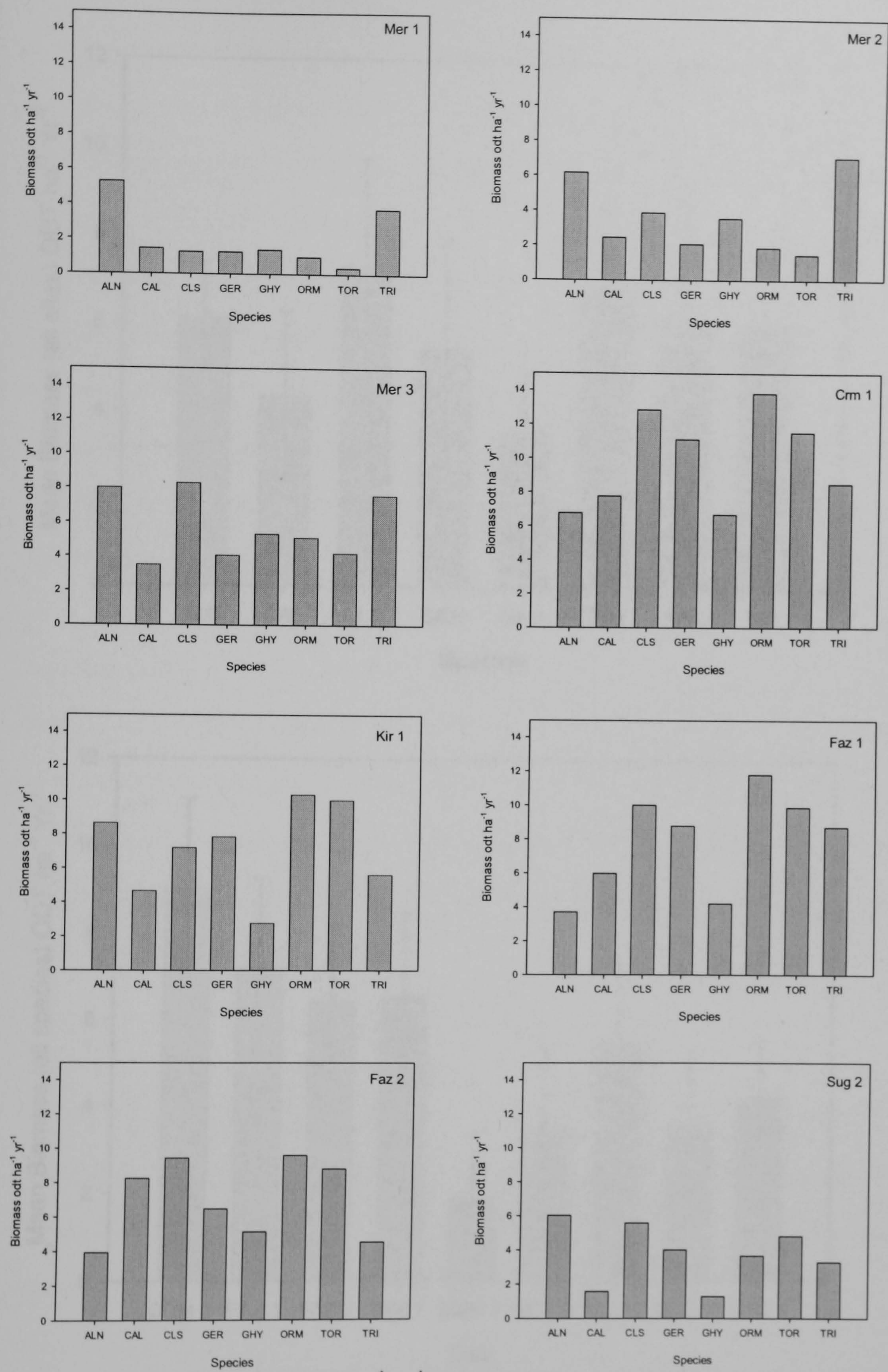


Figure 6.8. Total biomass in ODT ha^{-1} yr^{-1} after two full growing seasons for all species shown by site

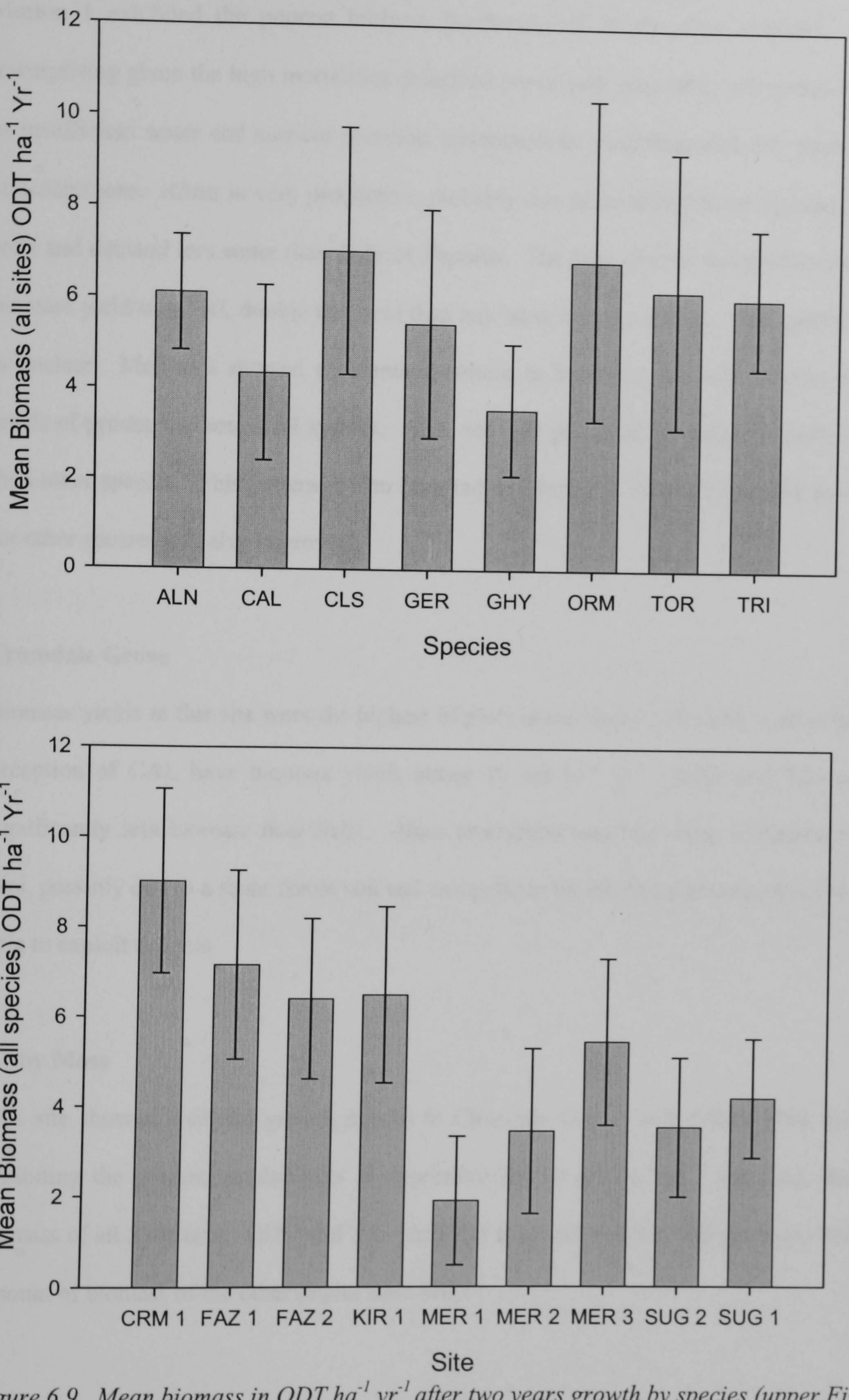


Figure 6.9. Mean biomass in ODT ha^{-1} yr^{-1} after two years growth by species (upper Figure) and by site (lower Figure). Error bars represent 95% confidence interval.

Merton Bank

Merton 1 exhibited the poorest biomass production of all the sites assessed. This is unsurprising given the high mortalities described previously, plus other site constraints such as insufficient water and nutrient retention, unstable soils, vandalism and very high Arsenic concentrations. *Alnus* is very productive, probably due to its ability to fix nitrogen in poor soils and demand less water than *Salix* or *Populus*. The only species that produced a higher biomass yield was TRI, double the yield than any other coppice species. The reason for this is unclear. Merton 2 showed an identical pattern to Merton 1 but with slightly elevated levels of production across all species. ALN and TRI produced significantly more biomass then other species. This pattern is also repeated on Merton 3, although biomass production for other species was also improved.

Cromdale Grove

Biomass yields at this site were the highest of plots in the study. All *Salix* species with the exception of CAL have biomass yields above 10 odt ha^{-1} yr^{-1}. GHY and TRI produce significantly less biomass than *Salix*. *Alnus* production was less when compared to *Salix* taxa, possibly due to a more fertile soil and competition by the faster growing willows better able to exploit this site.

Kirby Moss

This site showed a similar growth pattern to Cromdale Grove, with ORM, TOR and CLS exhibiting the greatest productivity at approximately 10 odt ha^{-1} yr^{-1}, and CAL the least biomass of all *Salix* taxa. GHY and TRI yield less than willows, but TRI produced twice the amount of biomass of the other poplar taxa, GHY.

FAZ 1

The same broad picture was apparent for ORM, TOR and CLS, with the highest yield figures at around 10 odt ha^{-1} yr^{-1}, and CAL producing the least biomass of the *Salix* taxa. However, TRI made significant improvements on this plot to show yields figures within 1-2 odt ha^{-1} yr^{-1} of TOR and CLS. Alder production was, however, low at this site.

FAZ 2

FAZ 2 is described later in this chapter due to different experimental treatments on the plot.

SUG 2

Overall SUG 2 showed low biomass production compared to other experimental plots. CAL still showed the lowest biomass production of *Salix* taxa and CLS and TOR the most, although in this case only just above 6 odt ha^{-1} yr^{-1}.

Ranking Biomass

Ranking the yields for each site, shows the position of each species within each plot based on total biomass figures (Figures 6.8 and 6.10).

Table 6.2. Ranked biomass for each species / taxa ranked within each site. 1= largest biomass, 8=least biomass

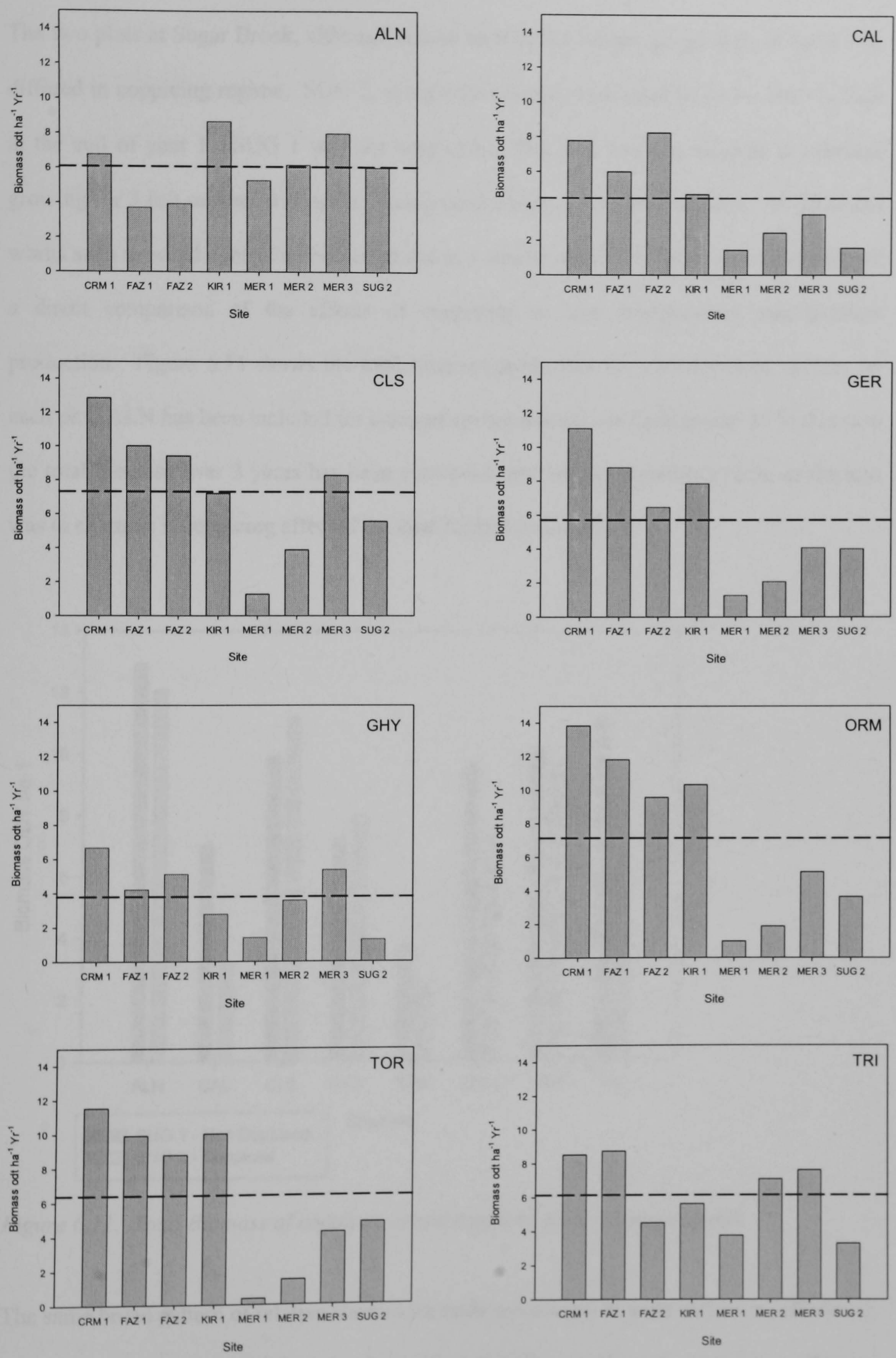
	MER 3	MER 2	MER 1	CRM 1	KIR 1	FAZ 1	FAZ 2	SUG 2
ALN	2	2	1	7	3	8	8	1
CAL	8	5	3	6	7	6	4	7
CLS	1	3	5	2	5	2	2	2
GER	7	6	6	4	4	4	5	4
GHY	4	4	4	8	8	7	6	8
ORM	5	7	7	1	1	1	1	5
TOR	6	8	8	3	2	3	3	3
TRI	3	1	2	5	6	5	7	6

Summary

The mean biomass produced per species and per plot is shown in Figure 6.9 and ranked in Table 6.2. Overall the highest biomass yielding taxa were CLS and ORM, closely followed by TOR and ALN. TRI was the highest yielding poplar. However, as can be seen by the error bars, the variation in all willow production was large, reflecting site effects described previously. The variance for poplar and alder was much less, reflecting less extreme variation due to environmental conditions.

Figure 6.10 shows biomass production for each species across all sites, allowing a comparison of site variation in biomass production of individual species or taxa. The dashed black line represents the mean production for that species calculated across all sites.

Figure 6.10. Total biomass production of each species / taxa in ODT ha^{-1} yr^{-1} after 2 full growing seasons. The dashed line represents the species average from all sites.



6.6 Differential coppicing regimes – Sugar Brook

The two plots at Sugar Brook, although almost identical in design, preparation and planting, differed in coppicing regime. SUG 2, along with all other experimental plots, was cut back at the end of year 1. SUG 1 was not coppiced at this time and was allowed to continue growing for 3 full seasons with no coppicing conducted until the final harvest. All other site works such as weed control were carried out in a similar manner for each plot. This allowed a direct comparison of the effects of coppicing on tree establishment and biomass production. Figure 6.11 shows the total biomass production for each coppiced species on each plot, ALN has been included for comparison but was not cut back at year 1. In this case the total biomass over 3 years has been calculated and not the annualised yield, as the aim was to examine if coppicing affected the total initial harvest yields.

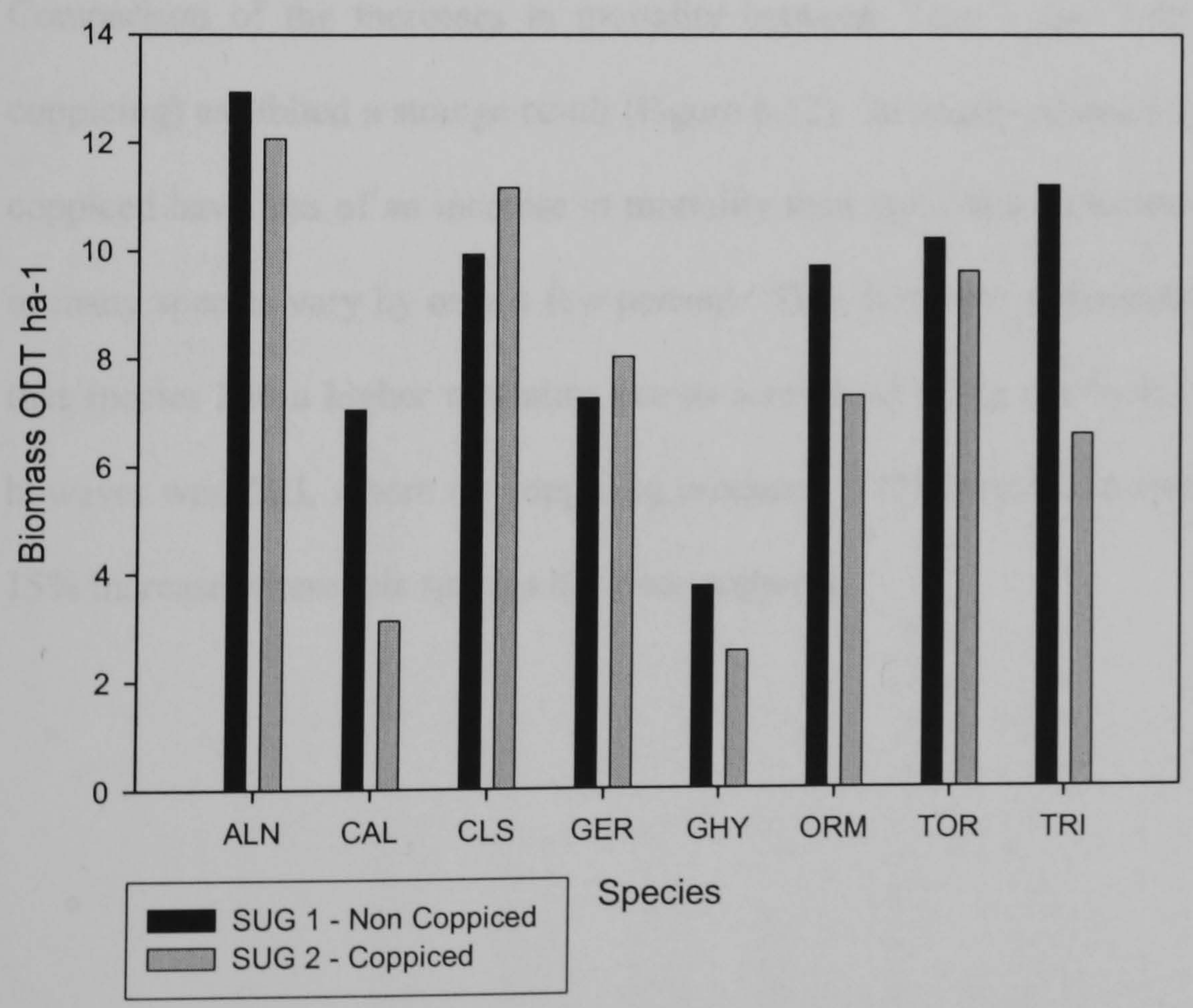


Figure 6.11. Total Biomass of coppiced vs non coppiced plots on Sugar Brook

The same broad pattern of relative success for each species can be seen here as is reflected in the ranking exercise in Table 6.2. It is clear that the species not coppiced performed marginally better than those that were coppiced (in all but 2 cases). The two exceptions were CLS and GER, but the additional biomass gained by coppicing was small, only 1 odt ha^{-1} or less. The most pronounced difference was in TRI and CAL taxa, where not coppicing led to almost a doubling of biomass. Poplars do not respond in the same way as willows to cutting back. Many poplar species show strong apical dominance and cutting back may only produce two or three main stems, which may be insufficient to shade out weeds (Tubby and Armstrong 2002) and compete with *Salix* taxa if grown in a mixture. Research suggests it may be beneficial not to coppice poplars until the first full harvest (Laureysens *et al.* 2003) this data seems to be corroborated with the results from this study. CAL would seem to be a slower growing taxa as it consistently produces less biomass on many plots (Fig 6.11). In this case coppicing has reduced its ability to compete with other species and taxa from re-growth, a lack of coppicing however, allows comparable growth rate with other taxa.

Comparison of the increases in mortality between Year 1 and Year 2 (before and after coppicing) exhibited a strange result (Figure 6.12). In nearly all cases species that have been coppiced have less of an increase in mortality than those that have not, although differences in many species vary by only a few percent. This, however, contradicts earlier suppositions that species had a higher mortality rate as a result of being cut back. A notable exception however was TRI, where no coppicing produced a 1% increase in mortality compared to a 15% increase where this species has been coppiced.

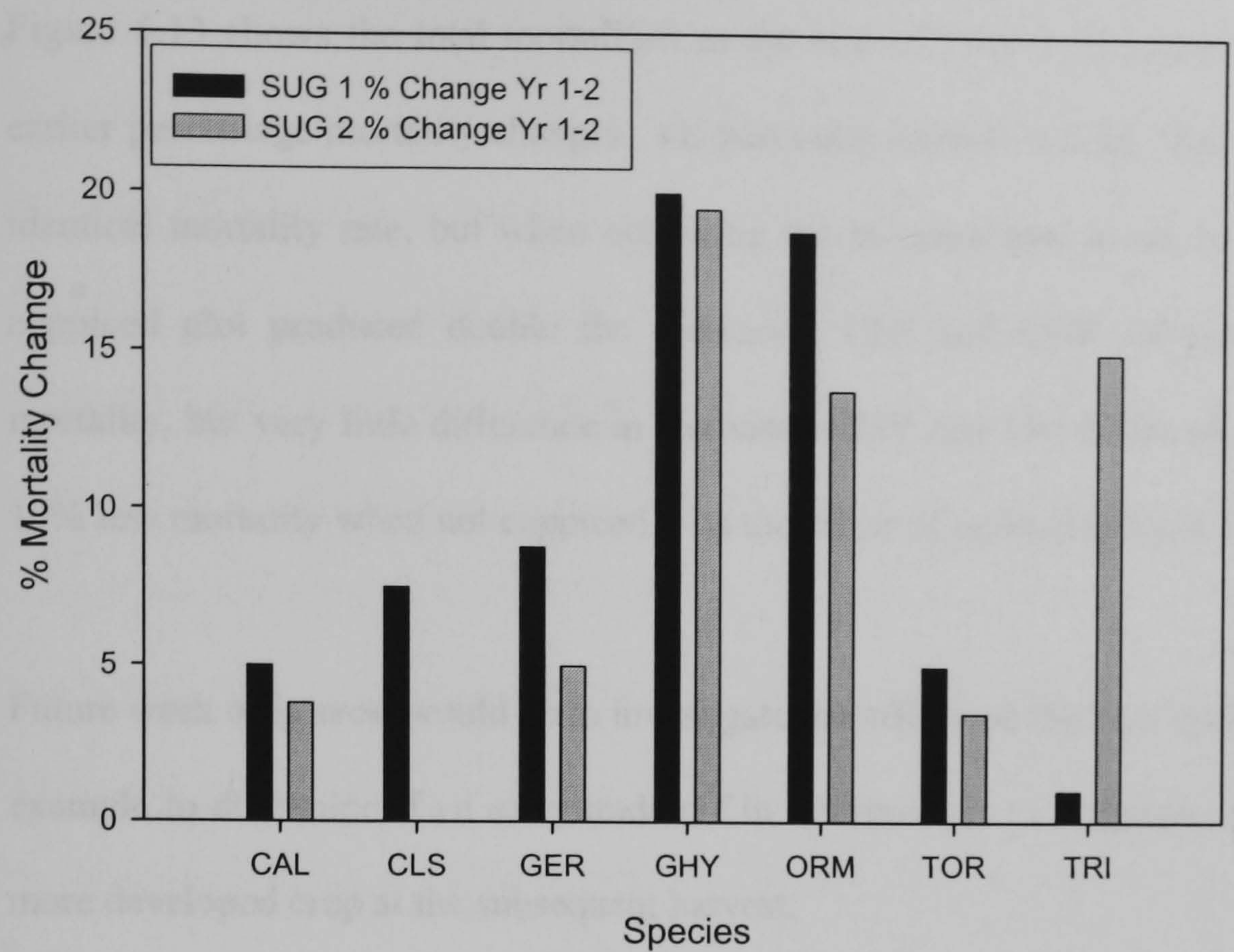


Figure 6.12. Percentage mortality change from Year 1 to year 2 (before and after coppicing) on Sugar Brook.

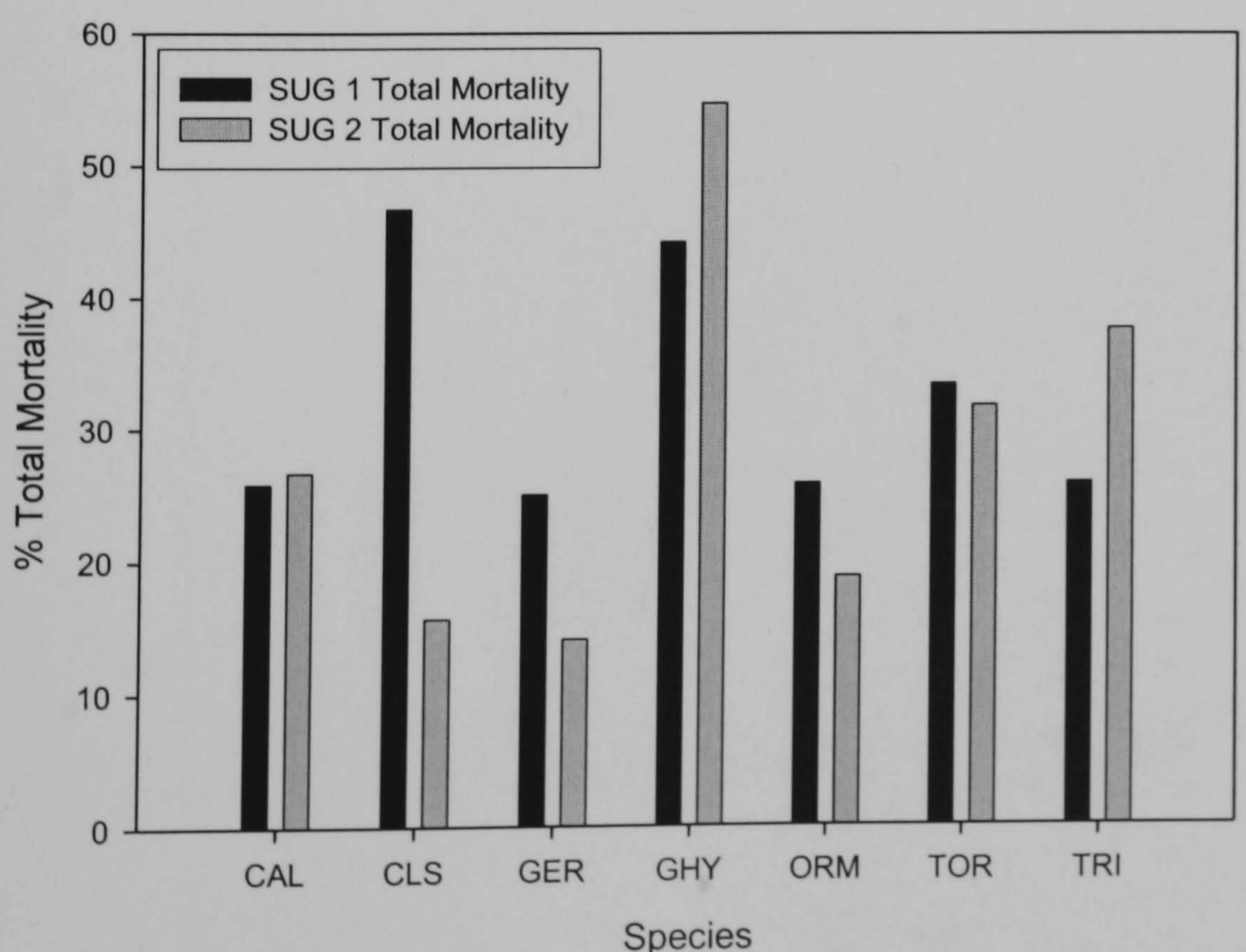


Figure 6.13. Percentage total mortality on Sugar Brook at end of Year 3

Figure 6.13 shows the total mortalities at the end of Year 3 and these broadly reflect the earlier percentage mortality changes. Of particular interest is CAL, this taxa had an almost identical mortality rate, but when observing the biomass data it can be seen that the non-coppiced plot produced double the biomass. CLS and GER showed large changes in mortality, but very little difference in biomass. GHY and TRI followed a similar pattern of 10% less mortality when not coppiced with the effect of producing more biomass.

Future work of interest would be to investigate the effects on the next cycle of harvesting, for example to determine if an early trade off in biomass due to coppicing provides a stronger more developed crop at the subsequent harvest.

6.7 Soil Amendment experiment – FAZ 2

The plot FAZ 2 was constructed to test the effects of different soil amendments and how they may influence the uptake of metals or affect tree growth. Full experimental details can be found in Chapters 3 and 4. To summarise, the plot was sub-divided into 3 replicate treatments, each with a different amendment incorporated into the soil. The first was treated with sewage sludge at an equivalent rate of 595 tonnes ha^{-1}, the second with dried sewage cake at an equivalent rate of 595 tonnes ha^{-1} and the third received no amendment. Each treatment was planted with the same block design and number of species. The influence of these treatments on the cycling of heavy metals is discussed in Chapter 5. Figure 6.14 shows the biomass production figures for the 3 treatments, together with the pooled figures for the entire plot.

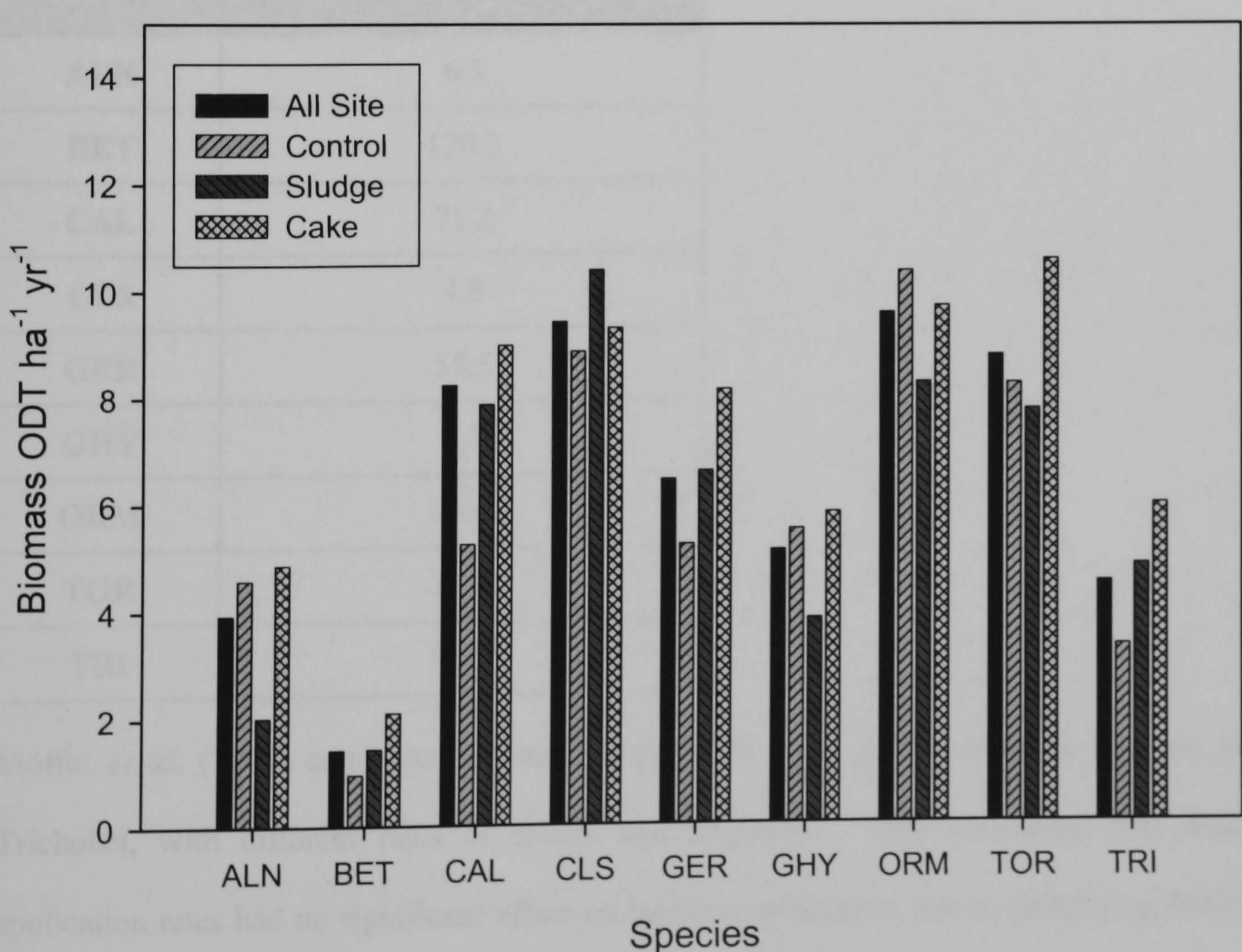


Figure 6.14. Total biomass in ODT ha^{-1} yr^{-1} after 2 growing seasons for each species and amendment treatment at FAZ 2

The overall response to the treatments showed that, in 7 of the 9 species tested, the addition of sewage cake increased biomass production over the effect of adding sewage sludge or no amendment. For 4 species tested (ALN, GHY, ORM, TOR) the addition of sewage sludge caused a decrease in biomass from the control (no amendment) treatment. Visual observations of the plot suggest this was due to the difficulty of incorporating sludge evenly. Large areas of sludge were not fully incorporated and the land became waterlogged or detached from the soil. Due to the drier, more friable nature of sewage cake it was more easily incorporated into the soil. This site was prone to natural waterlogging and therefore the addition of sewage sludge may have exacerbated this problem in places.

Table 6.3. Percentage increase or decrease in yield comparing control treatment (no amendment) to amendments using sewage cake on FAZ 2.

Species	% change in yield from control using sewage cake
ALN	6.3
BET	120.3
CAL	71.2
CLS	4.9
GER	55.5
GHY	5.7
ORM	-6.4
TOR	28.4
TRI	80.8

Moffat *et al.* (2001) conducted a similar experiment using poplar cultivars Beaupre and Trichobel, with different rates of sludge and irrigation. They concluded that sludge application rates had no significant effect on biomass production, due to underlying fertility of the soil, but that irrigating the trees had a positive effect on yield. The non irrigated clones of TRI yielded between 2.19 and 4.13 odt ha^{-1} yr^{-1}(n=3), compared to 4.81 and 5.96

odt ha^{-1} yr^{-1} for sludge and cake treatments respectively on the FAZ 2 plot in the present study. Similar experiments also conclude that with the addition of soil amendments, water is more of a limiting factor than nutrients (Hasselgren 1998). In the present study water was not a limiting factor due to wet summers and the low lying nature of the plot that was frequently waterlogged. Experiments by Adegbidi *et al.* (2003) concluded that a high rate, single application of lime-stabilised sewage sludge was more effective to increase yield production than stands amended with traditional slow release fertilisers such as Osmocote. This is significant as inorganic fertiliser addition suggested by DEFRA (2002a), constitutes a considerable expenditure. It also allows for a single application of sewage amendment, an option supported by several studies, as repeat applications to a mature crop of SRC are operationally problematic.

The fertiliser potential of sewage products for forestry is well supported (Wolstenholme *et al.* 1992). Use of organic amendments in forestry has mainly been in restoration schemes where organic matter has been lost due to mining operations (Bending and Moffat 1999; Rate *et al.* 2004) or on substrates with naturally low nutrient supply, such as spoil heaps. Its use in SRC production is also receiving considerable attention (Riddell-Black 1995; Hasselgren 1998; Nixon *et al.* 2001). Many experiments have concentrated on the ability of short rotation coppice to remediate land contaminated by historic sewage sludge application (Pulford *et al.* 2002) whist others have advocated additional sludge application, harnessing the increased nutrient status of such soils to produce increased commercial yields on otherwise non productive sites (Steer 1997; Bardos *et al.* 1999).

The present study, supported by others cited above, suggests that correctly applied and incorporated sewage cake can increase biomass yield in a majority of *Salix* and *Populus* species. However, the issue remains of introducing heavy metals inevitably present in sewage sludge and their subsequent ecosystem mobility. This topic is discussed in Chapter 5.

6.8 Discussion

Many different biotic and abiotic factors affect establishment and growth of coppice, including climate, pests and weed competition. These are beyond the scope of this investigation, but the close geographical proximity of these sites and consistent nursery stock for site planting, preparation and maintenance has helped to negate or balance these issues.

Overall mortality of willows was low compared to other studies on agricultural soils, perhaps due to the use of polyclonal stands. Although the decision to plant 10 different species within each plot was governed primarily by the requirement for experimental diversity, there is strong evidence that growing coppice within a polyclonal environment is beneficial in terms of survival and yield (Dawson and McCracken 1995). McCracken *et al.* (2001) tested up to 20 different *Salix* taxa grown in mixtures and monoclonal plots, principally to reduce the transmission of rusts such as *Melampsora*. The experiment was successful in reducing fungal pathogen transmission and increasing biomass yield using mixtures over monoclonal plots. *Salix* grown in monoclonal plots with an absence of genetic diversity are particularly susceptible rust and other fungal and herbivorous attacks.

There may be an advantage in added biodiversity when the range of species or taxa used is increased (Perttu 1998). This experiment has also introduced additional diversity by growing species such as *Alnus*, *Betula* and *Larix* within a short rotation forestry system. Although larch and birch performed relatively poorly, mainly due to competition from faster growing coppice species, they will have added diversity and may have reduced or prevented transmission of disease and thus increased the yield of SRC species. In most cases *Alnus* has been a success in biomass production and survivorship grown alongside *Salix* and *Populus* coppice species. Soil nitrogen fixation associated with *Alnus* root nodules has been used in other schemes to act as a nurse crop and aid the growth of other species that lack nitrogen fixing bacteria grown on nutrient poor soils, although the utilisation of nitrogen may take up to 5 years to be significant (Moffat 2000).

From a risk management, phytostabilisation or community forestry perspective, the use of mixed plots can be strongly recommended, adding biodiversity and survivorship in the long term. However, if used on a commercial scale it may not be practical given commercial harvesting machinery set up for *Salix* harvesting and problems with the regrowth of alder, birch or larch from harvesting.

On average *Salix* taxa such as CAL and GER showed poorer biomass production, but also showed the lowest mean mortality over 3 years. It may therefore be possible to select taxa on a 'fit for purpose' approach where long term survival may be a more desirable trait than biomass production. Clearly such a decision making process may also include the amount of metal removed if phytoextraction is desired, this is discussed in Chapters 5 and 7. Of future interest would be subsequent sets of harvest data, to determine whether lower mortality leads to higher growth and biomass production in future seasons.

Biomass yield varies across all sites. For all *Salix* species a clear difference can be assigned between those growing on the MER and SUG sites, where yields fall below the average for all sites, and the CRM, FAZ and KIR sites where average yields are all above the experiment average (Figure 6.15). Average yields of short rotation forestry across central and northern Europe are estimated to be approximately 8-12 odt ha^{-1} yr^{-1} (Makeschin 1999), although this may assume some degree of fertilisation or irrigation, which has not been undertaken on many of the experimental plots in this study. Based on funding and economic return considerations a yield of around 8 odt ha^{-1} yr^{-1} (Mitchell et al. 1999) to 10 odt ha^{-1} yr^{-1} (Tubby and Armstrong 2002), is desirable to provide a viable economic return, although initial yields in the range of 7-12 odt ha^{-1} yr^{-1} should be considered acceptable (DEFRA 2002a). An average baseline figure for biomass production of 10 odt ha^{-1} yr^{-1} has been reached or exceeded by 4 of the 5 species on Cromdale Grove, 3 of the 5 on Faz 1 and 2 of the 5 for Kirby Moss. Furthermore, many other species achieved a yield of 7.5 odt ha^{-1} yr^{-1}, approaching the 10 odt ha^{-1} breakpoint, and still within the acceptable limits for an initial harvest.

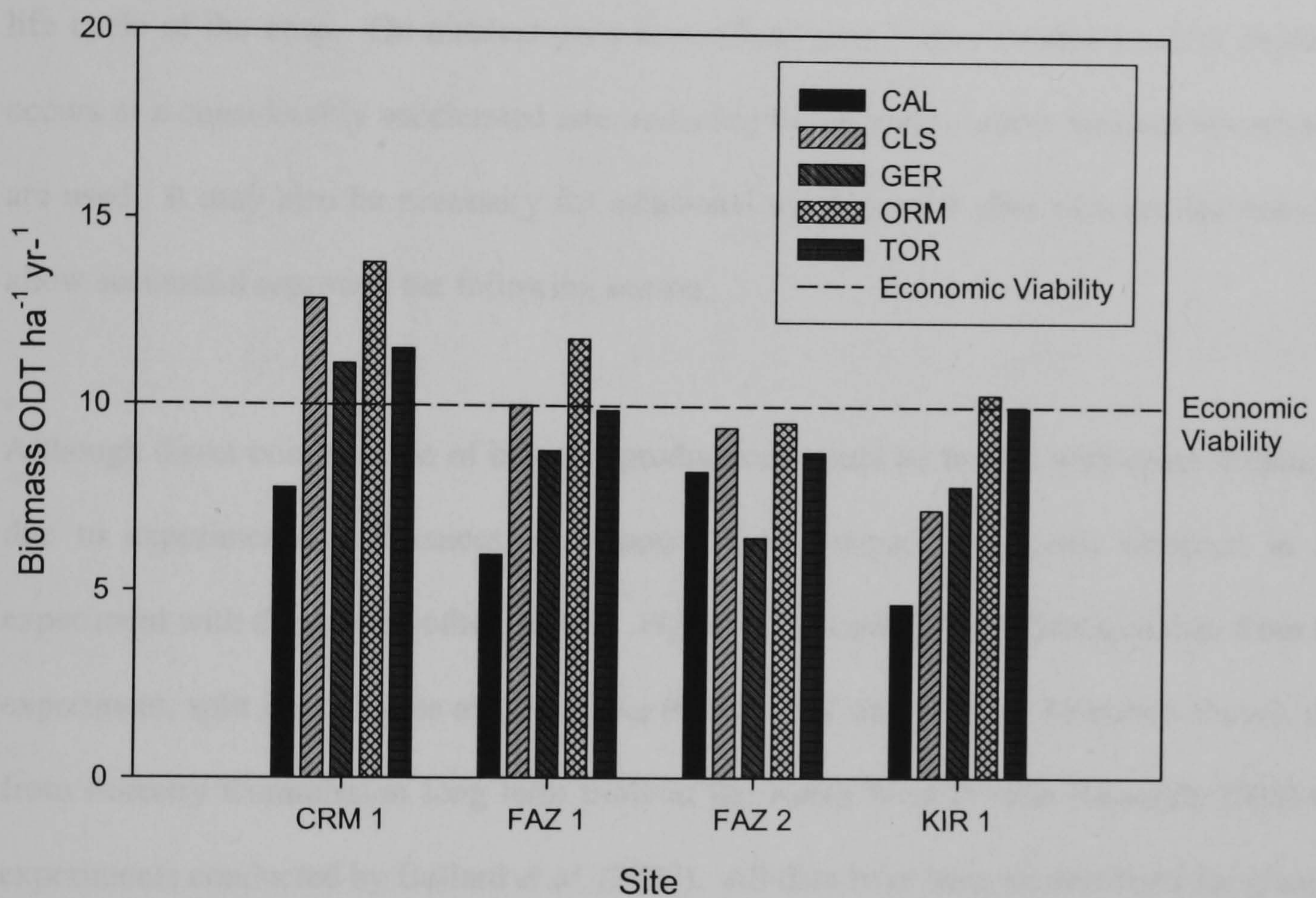


Figure 6.15. Biomass of selected clones in ODT ha^{-1} yr^{-1} (based on 2 year harvest) related to potential economic viability

Data collected as part of the Forestry Commission research programme into site / yield relationships (Tubby and Armstrong 2002) showed that, although demonstrating considerable variation, the yield for the second 3 year cutting cycle had increased from 8.8 to 18.22 odt ha^{-1} yr^{-1}, indicating that future harvests have the potential for much higher yields once a crop is established.

If similar patterns of harvesting were to be repeated at the next cutting cycle it is entirely possible that all the willow species grown on the 'best' sites CRM, FAZ and KIR would exceed the 10 odt ha^{-1} yr^{-1} figure, as well as several species growing on the MER sites, showing potential commercial viability. However, species not reaching this threshold should not necessarily be discounted from use, depending on the perceived end use (i.e: non-commercial), lower yields could be tolerated should the site be for a landscape, amenity, community or risk management goal where survivorship would be of more importance. Of concern however, is the sustainability of continued harvesting over a traditional 15 - 20 year life cycle of the crop. On nutrient poor brownfield sites it may be that nutrient depletion occurs at a considerably accelerated rate, reducing future yields unless nutrient amendments are used. It may also be necessary for additional weed control after sites are harvested, to allow successful regrowth the following season.

Although direct comparisons of biomass production should be treated with some skepticism due to experimental differences, it is possible to compare the yields obtained in this experiment with those from other studies. Figure 6.16 shows average biomass data from this experiment, split into all sites and best sites (CRM, FAZ and KIR, as described above), data from Forestry Commission long term trials in the North West (Forest Research 2003) and experiments conducted by Bullard *et al.* (2002). All data have been standardised for planting density, harvest frequency and species as far as possible, but where these differ it is indicated.

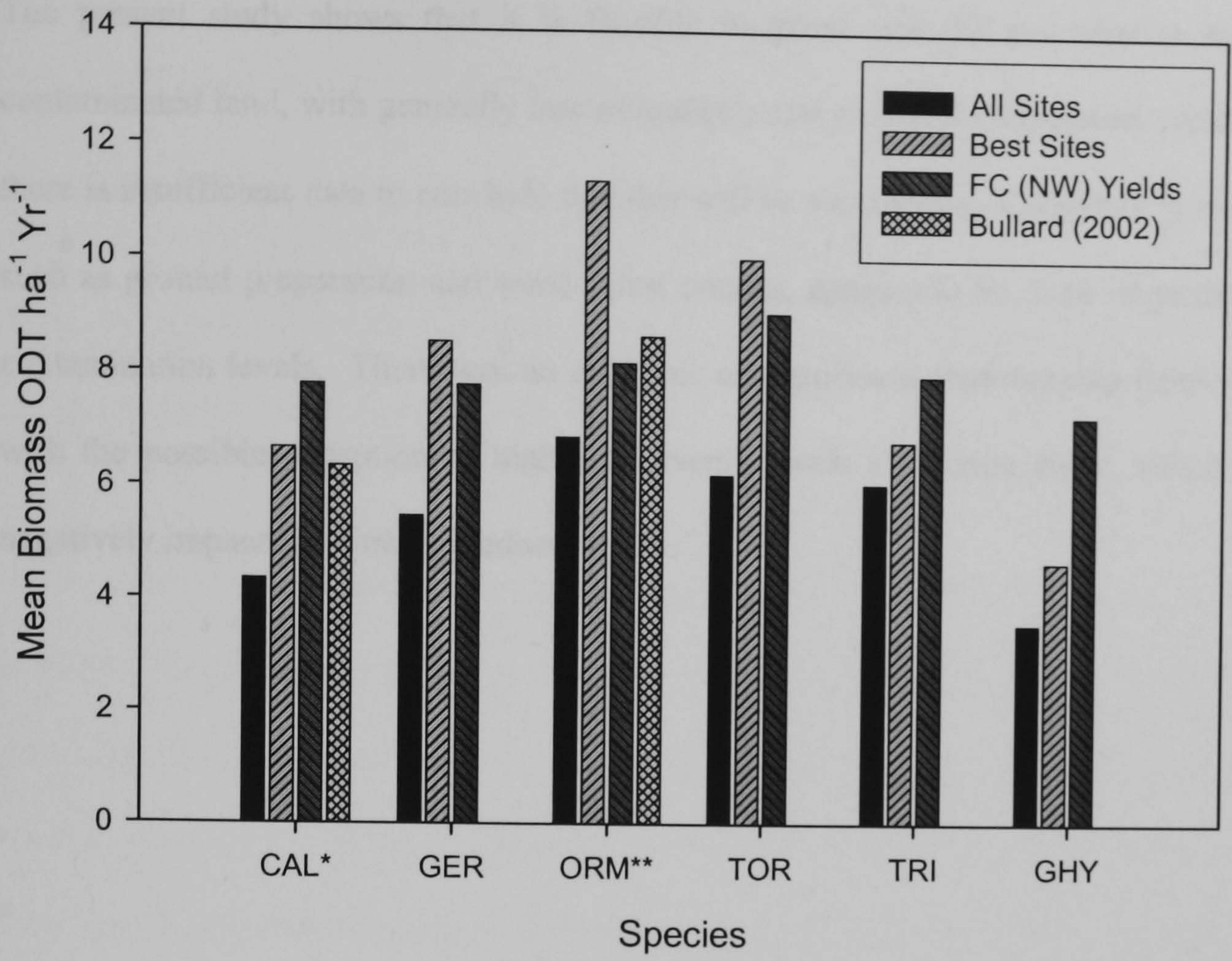


Figure 6.16. Comparison of biomass production in this study to that achieved in different experiments.

* Data from FC and Bullard is from *Salix dasyclados*, a similar parentage taxa (cap x cin x vim) to CAL

** Data from Bullard is for *Salix viminalis* cv. Journn, a similar parentage taxa (vim x vim) to ORM

The main objective of Figure 6.16 is to illustrate that yields, certainly on the better sites, compare favorably with those grown in other studies. *Alnus* yields were high across most sites, matching short rotation taxa on many occasions. Although data for *Alnus* grown in short rotation systems is rare, the best yields obtained by Steer (1997), were 2.93 odt ha^{-1} yr^{-1}

New, higher planting densities of between 23,700 – 111,000 plants ha^{-1} now being introduced for SRC can increase biomass yield for certain species, and shorter harvest intervals, such as biennial harvests will increase biomass (Bullard *et al.* 2002). However, the advantage of this may be negated if polyclonal mixtures are used at denser spacing and this work is based on fertilised agricultural land, not brownfield sites where higher planting densities may not be sustainable.

The present study shows that it is feasible to grow selected tree species and taxa on contaminated land, with generally low mortalities and potential commercial yields, although there is insufficient data to conclude that this will be sustainable. Correct crop management, such as ground preparation and weed / pest control, appears to be more important than soil contamination levels. There was no evidence of significant phytotoxicity from soil metals, with the possible exception of high soil arsenic levels at Merton Bank, which may have negatively impacted biomass production.

Chapter 7: Predictive Uptake Models

7.1 Introduction and methodology

In previous chapters the extent to which individual varieties or taxa accumulate metals in their above ground-tissues (Chapter 5) and their biomass production over a 2 year growing period (Chapter 6) has been investigated.

Different phytoremediation strategies may require specific attributes of a tree species. For example, phytoextraction requires high biomass and high uptake, (predominantly into stem), commercial biomass production requires high biomass and low metal uptake and community forestry requires low mortality, moderate growth and biodiversity. To accurately predict the long term field potential of these differing strategies it is necessary to collate data in order to examine the actual quantity of metals removed at harvest, and those removed from or left in the soil. Such extrapolations must take into account factors such as seasonal leaf fall that may potentially return metals to the soil surface or allow dispersal into the wider environment.

Previous studies have used similar techniques to estimate the amount of time required to remove a given quantity of contaminant from a contaminated soil (Riddell-Black 1994; Pulford *et al.* 2002; Vervaeke *et al.* 2003). Such studies, through necessity, are frequently based on a number of assumptions in what is inherently a temporally and spatially variable biological system. Extrapolations made in the present study are based on real-time field data collected over a 3 year period.

7.2 Extrapolations

When extrapolating uptake and yield data, several assumptions were made (Table 7.1).

Table 7.1. Summary of assumptions used for biomass predictions in the present study

Assumption	Description
Use of average yield for each species.	Although this is calculated from replicate blocks, variability may be high within species, even within a small plot.
Use of average uptake figures for stem and foliage	These uptake figures may vary within species or taxa and within a small plot.
Using ODT ha^{-1} figures per species / taxa	Assumptions are made as if 1ha was planted with the same taxa, and not in a mix as occurred in the experiment. Growing large areas of monoclonal trees may reduce yield, for example due to rapid pest transmission or nutrient depletion. Conversely successful growth of the most suitable taxa may increase yield.
Using foliar biomass data	Calculations of foliar biomass were based on research conducted elsewhere and from literature sources.
Consistency of contaminant availability and uptake rate over a 3 year period or longer	Soil conditions (e.g. pH) change over time, potentially changing soil contaminants available for uptake. Soil conditions and contaminants are assumed homogeneously dispersed, which is frequently not the case (Chapter 4).
Growth Rate	Growth rate over 2 years is assumed to be sustainable and constant.
No major physiological impediments to tree growth	e.g.: drought, sudden change in soil conditions (pH, waterlogging, nutrient leaching etc), major pest attack etc.

7.3 Calculating Leaf Biomass

No field measurements were made during this study of leaf biomass as a constituent part of total above-ground biomass. Stems were harvested in the winter when no leaves were present.

Few studies have been conducted into the proportion of above ground biomass that is a result of foliage. Maxted (2002) discovered 15% of total above ground biomass was the result of foliage in *Salix caprea x cineria x viminalis* (the same parentage as CAL used in this study). Klang-Westin and Eriksson (2003) reported a range of 17 – 37% of total above ground biomass attributable to foliage in taxa of *Salix viminalis*. Kurth Perttu *(pers. comm.)* cited by Greger and Landberg (1997) reported 30% of total above ground biomass in the foliage. A recent Scandinavian study (Perttu 2002) reports 25% (+/- 10%) of above ground biomass is derived from foliage. Therefore a conservative leaf weight ratio (LWR) estimate of 20% was adopted for the present study, based on the literature cited, when extrapolating and calculating total leaf metal concentrations in *Salix*. Whilst consistent with other studies, these figures have been obtained predominantly from *Salix viminalis* and there are likely to be morphological differences amongst taxa that will affect the proportion of leaf biomass.

Studies on the biomass of *Populus* foliage are also rare. A recent study by Pellis *et al.* (2004) examined 17 poplar taxa and calculated leaf weight ratios (LWR) based on field sampling. These ranged from 31 – 44%. The LWR for TRI was 36%, and this figure has been adopted for extrapolation in this study. No direct measurement was made for GHY, although assessments were made for 3 *Populus deltoides x nigra* taxa, the same parentage as GHY, and ranged between 32 – 35%. A mean value of 33% has been used as the LWR for GHY in this study.

7.4 Harvesting Systems

Leaves accumulate more metals towards the end of the growing season prior to leaf senescence (Baker 1981; Vandecasteele *et al.* 2002). It may therefore be possible to harvest the trees before senescence occurs and remove metals contained within the foliage. Alternatively it may be beneficial to allow leaf senescence to occur and harvest the stems and sequestered metals using a more traditional harvesting technique. Some leaf loss is inevitable in intervening years between harvests and during the growth season. The advantage of harvesting trees with leaves intact is balanced by the fact that the harvest may occur before stems have completed their full annual growth, although additional secondary growth may occur if stems are harvested in July or August. Table 7.2 examines the advantages and disadvantages of these different coppicing regimes. Some of the technical aspects are discussed later in this chapter.

Table 7.2. Summary of advantages and disadvantages of different coppicing regimes to maximize metal removal

	Stems only	**Stems and Leaves**
Advantages	• Easier harvest using traditional means • Less precision and containment required • Easier post-harvest processing • Allows less frequent harvesting cycles	• Greater metal removal overall • Less metal dispersed into the environment • Potential for secondary growth
Disadvantages	• Total metal offtake is less • More metal dispersion into the environment	• May not achieve full season yield • Difficult to time harvest • Conventional harvesting techniques may be difficult • Drying, storage and processing made difficult by foliage

Extrapolations in the present chapter are used to quantify:

- Actual and theoretical uptake from plots using field data (Table 7.3), comparing species mixes used in the field trails with theoretical extrapolations based on the future use of monoclonal plots.
- A wider appreciation and investigation into models to predict total removal over the lifetime of the crop.

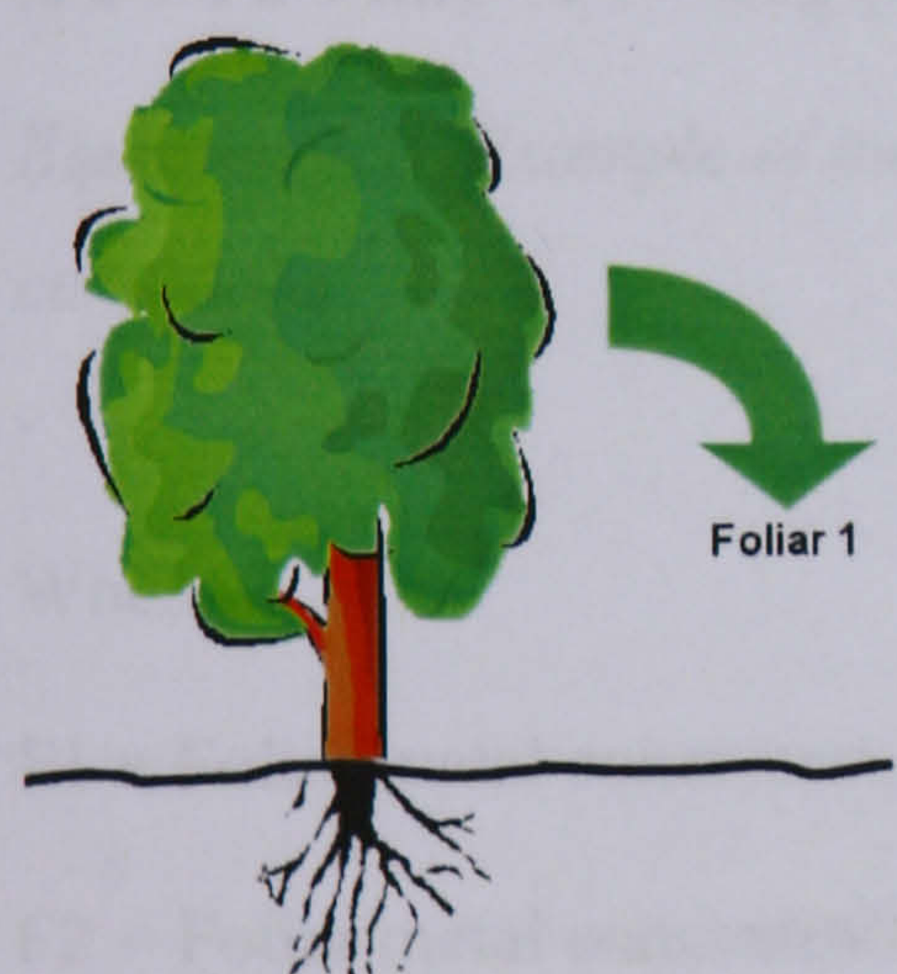
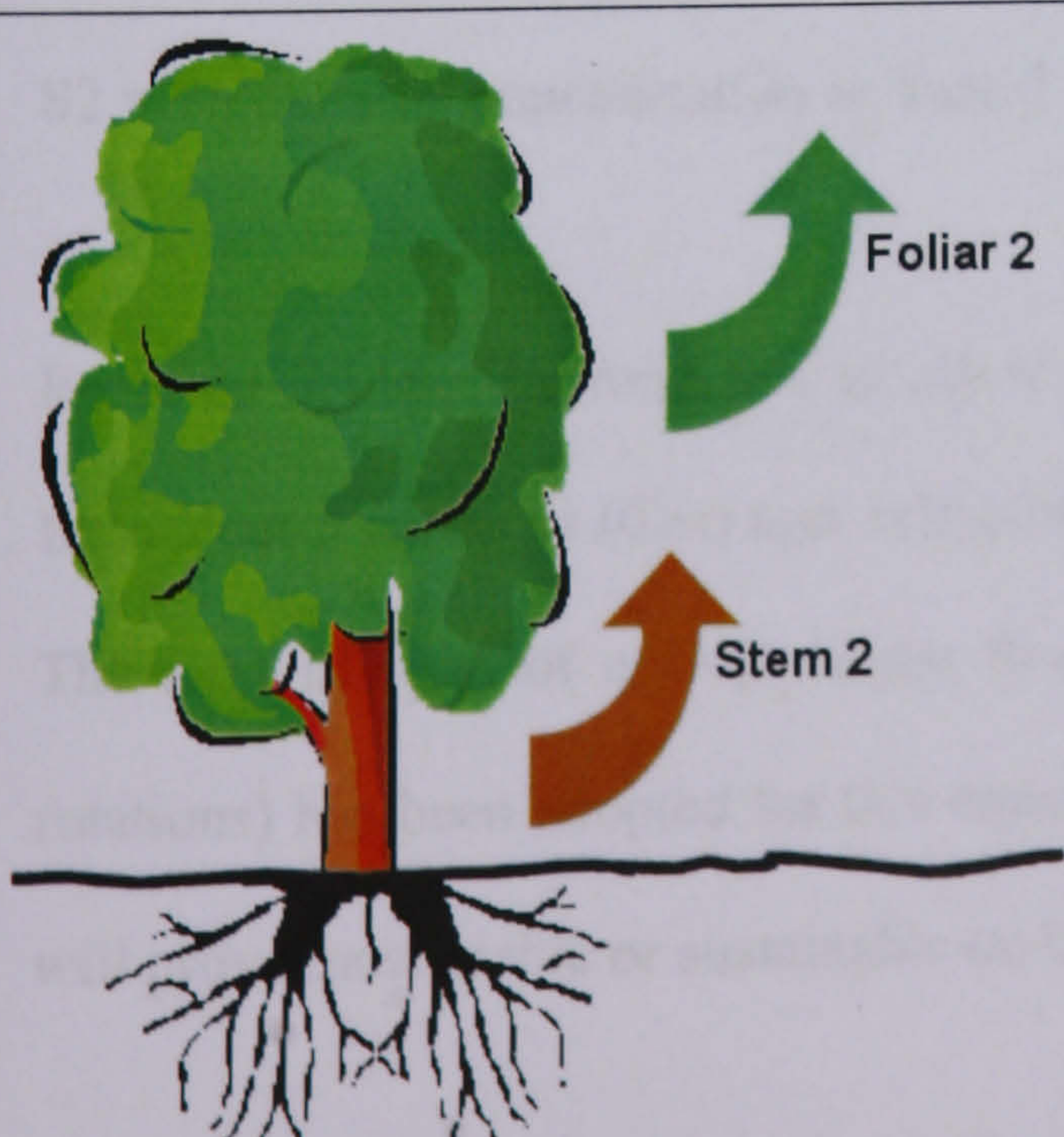
Table 7.3. Field measured parameters used for extrapolation and prediction of metal removal over the lifecycle of a crop.

Foliar metal levels from Year 2 (1^{st} full growing season)
Foliar metal levels from Year 3 (2^{nd} full growing season)
Stem metal levels from Year 3 (2 full years of growth)
Total stem biomass – from 2 years growth. Calculations using this data show: • ODT ha^{-1} yr^{-1} giving annulised yield figures • Biomass of foliage (utilizing figures in section 7.3)

7.5 Models of Uptake

Figure 7.1 shows how these data have been used to derive estimates and extrapolations of the metals removed during the study. The worked example in Figure 7.1 is for CAL uptake of Cd on CRM 1, but the same equation is applied to subsequent calculations showing metal fluxes to and from the soil. In this example, concentrations have been extrapolated to show uptake based on field data scaled to 1 ha.

Figure 7.1 Model used for extrapolations of total metal removed and returned to the soil

	Time	**Soil Input**	**Soil Output (Plant extraction)**
	**Year 0 (establishment and cut back)**		Cutback Stems (no foliage data is quantified in this study)
	**Year 1**	Foliar metal conc x foliar weight (F1) = 11.1g Cd	
	**Year 2**		Foliar metal conc. x fol wt = (F2) 17.8g Cd Stem metal conc x ODT stem wt = (S2) 43.7g Cd

Footnote to Table 7.1

Year 0 – cutting back stems will produce additional metal removal from the system. It is unlikely that foliage will also be harvested as initial cutting back must be carried out in the dormant season to prevent secondary growth. However, biomass yield for cutbacks in this study were extremely low and are unlikely to form a significant part of metal extraction.

Year 1 – leaf fall at the end of the 1st full growing season will return metals in the leaves to the soil.

Year 2 – If harvesting is timed correctly then leaves and stems will be removed.

Thus the equation for removal of Cd in grams in a 2 year harvest based on growing 1ha of CAL on CRM 1 is:

(F1 + F2 + S2) – F1 = 62g (total Cd removed)

Equation 7.1. Example of individual element removal under optimum 2 year harvest conditions

Where

F1 = Foliar metal concentration in Year 1 (subsequently returned so soil through leaf fall)

F2 = Foliar metal concentration in Year 1 (removed in Year 2 harvest)

S2 = Stem metal concentration in Year 2 (removed in Year 2 harvest)

Equation 7.1 can be modified to allow for repeated harvesting, plus additional extraction from Year 0 cutbacks (CSt) and extracting the root bole at the end of the crops lifespan (Rt). The total lifespan of a crop ranges from 15 – 30 years. A 20 year cycle (10 x 2 year rotations) has been adopted for this equation, although the number of repeated harvests that will prove most viable or sustainable on brownfield land is presently unknown.

These additional parameters can therefore be considered and amended to Equation 7.1 as follows, to allow calculation of long term extrapolated uptake:

CSt = Cutback stems (Year 0) total metal concentration

Rt = Root Biomass total metal concentration

10(F2 + S2) + CSt + Rt

Equation 7.2. Total theoretical metal removal over a 20 year (10 x 2 year harvest) timeframe

No data were collected during this experiment on either biomass of the root system, or metal concentrations in the roots. Only a handful of other studies have investigated root dynamics and have estimated that roots could extract a significant amount of metals, if not it is likely the roots would be removed to prevent remobilization of stored elements within it.

Values for species yield and metal uptake per plot have been averaged. Leaf biomass for *Salix* taxa is estimated at 20% and *Populus* at 33% (GHY) or 36% (TRI) (Section 7.3). No previous experimental work or literature review could be found that provided a leaf biomass value for, *Alnus, Betula* or *Larix* species and no field testing was conducted in the present study. Leaf uptake calculations have therefore not been formulated for these species.

The extrapolation models were applied used for a selection of contrasting sites, and data is presented for Merton Bank 3 (MER3), Cromdale Grove 1 (CRM1) and Fazakerley 2 (FAZ2).

7.6 Results

Figures 7.2 to 7.4 show the amount of metal contaminant each species would remove in above ground biomass if planted alone on 1ha and harvested after 2 full years of growth, in line with practices used in the present study.

The amount of contaminant removed was split into 3 groups:

1. Foliar 1 (F1) – The amount removed in the 1st full growing season, and that will be lost in leaf senescence and is therefore shown as a negative figure
2. Foliar 2 (F2) – The amount contained in the foliage after the 2nd full growing season. This quantity will be removed if harvesting occurs when the leaves are still on the tree.
3. Stem 2 (S2) – The amount contained within the stem after 2 growing seasons. This quantity will be removed irrespective of harvesting regime.

The calculations used to derive these values are discussed in section 7.5.

Figures 7.2 – 7.4 also show uptake results had the same species mix used in the experimental plots been planted on 1ha, and can be used as a baseline comparison. Further calculations made in tables 7.4 – 7.6 also use the same parameters described above.

Figure 7.2. Cromdale Grove 1 (CRM1) foliar and stem extrapolated uptake figures for 5 metals (g ha^{-1}) after a 2 year harvest

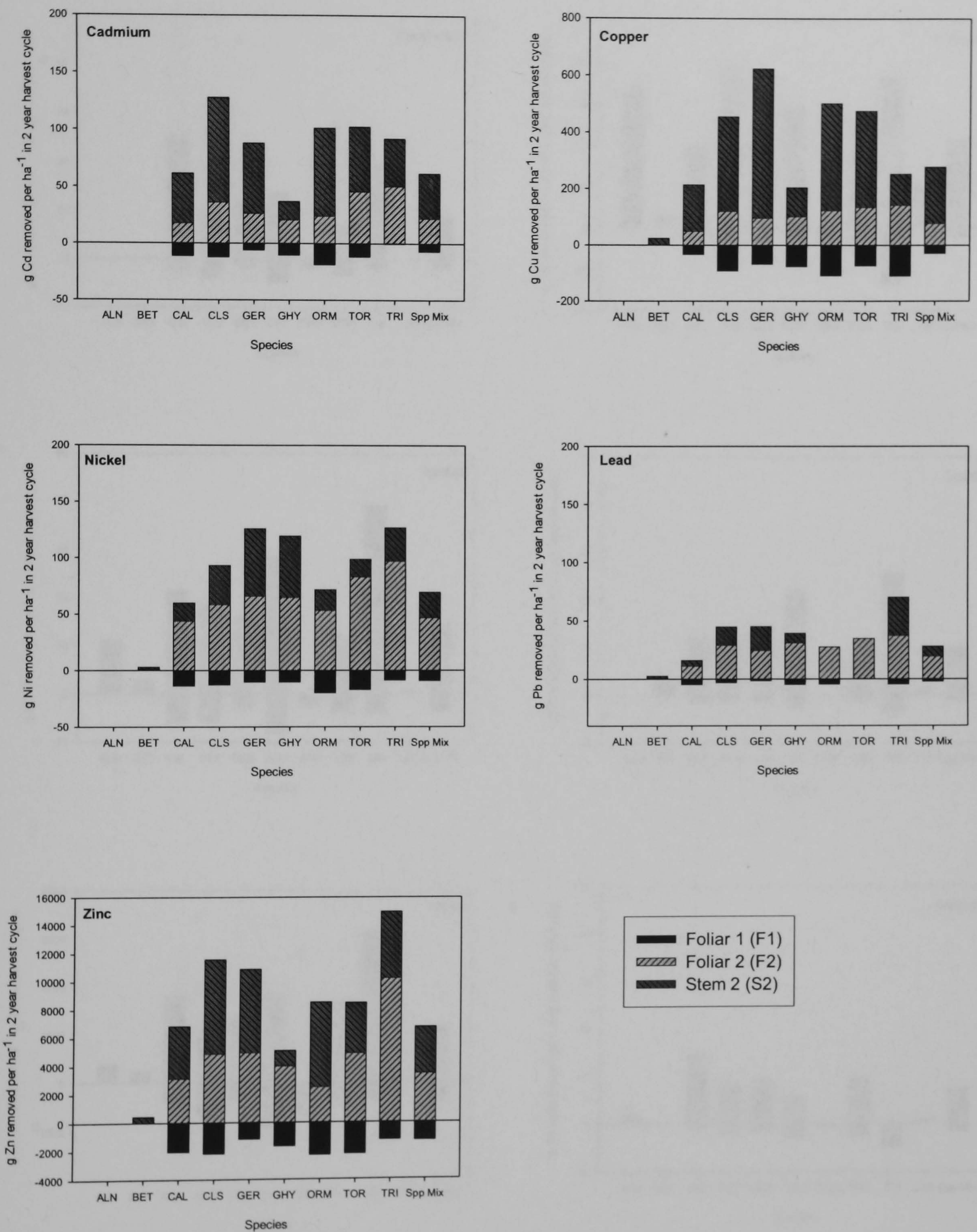


Figure 7.3. Merton Bank 3(MER3) foliar and stem extrapolated uptake figures for 5 metals ($g\ ha^{-1}$) after a 2 year harvest

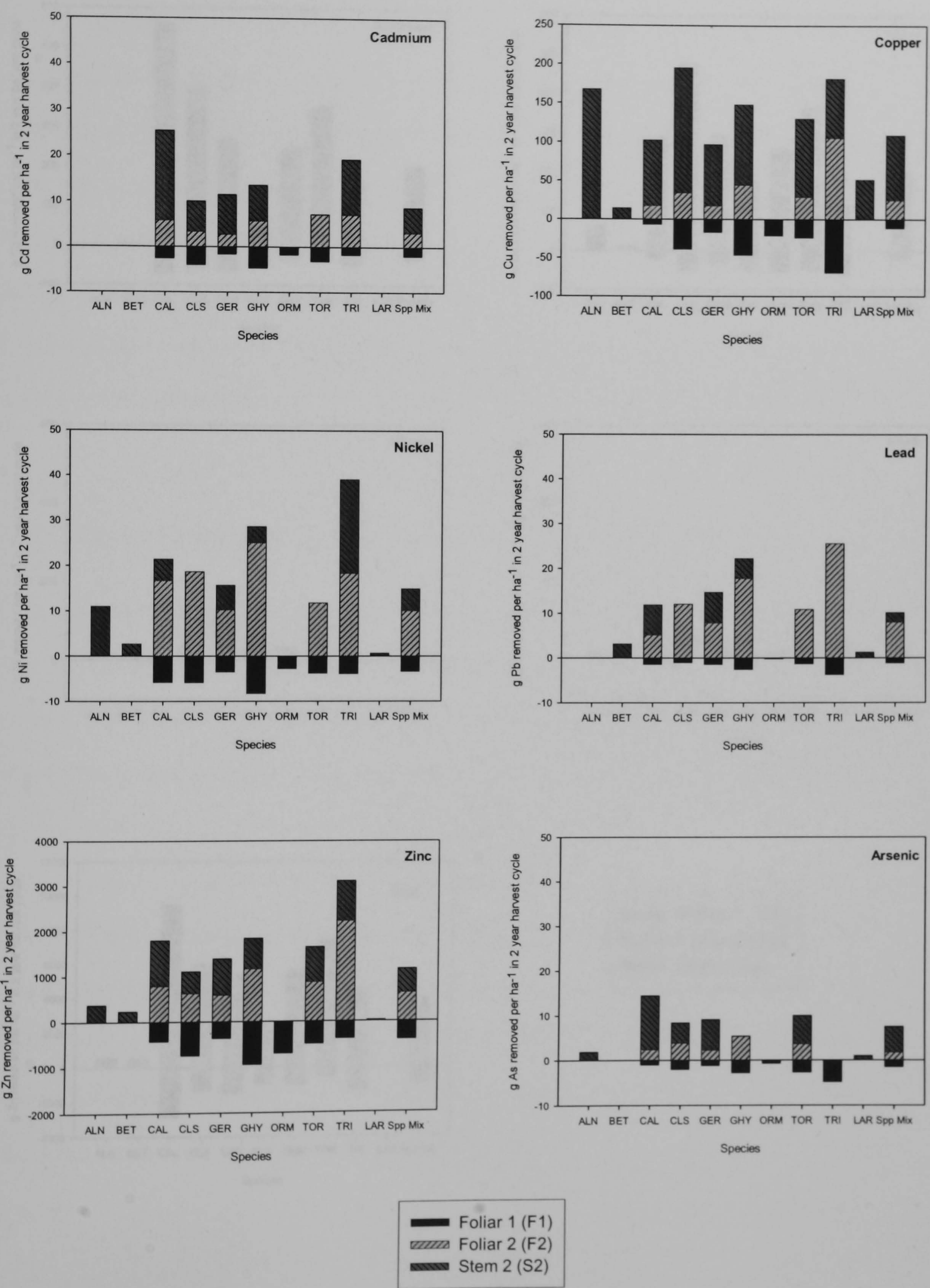
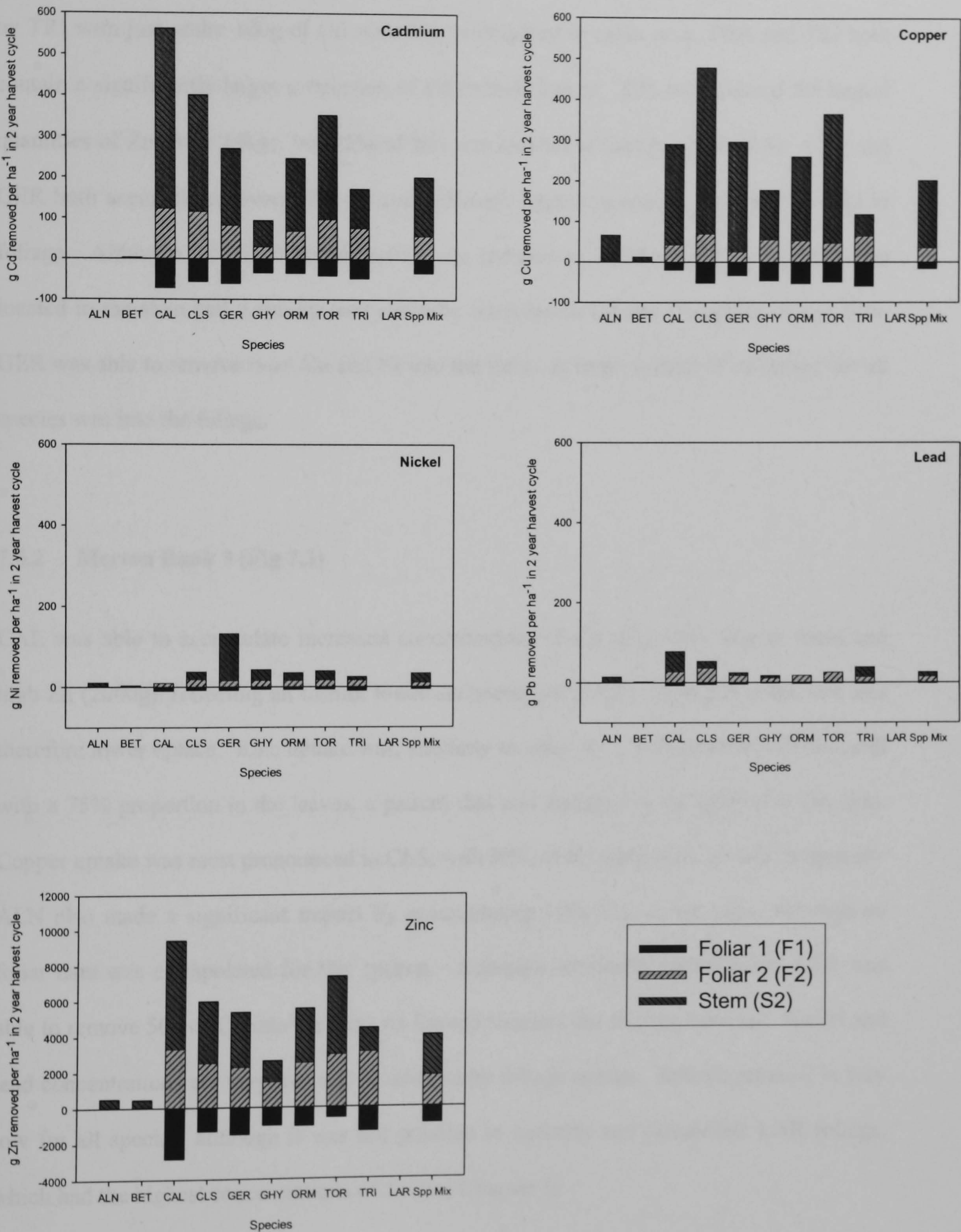


Figure 7.4. Fazakerley 2 (FAZ2) foliar and stem extrapolated uptake figures for 5 metals (g ha^{-1}) after a 2 year harvest

nb: The extrapolation for FAZ 2 is based on assessment of the plot as a whole, and not as separate treatments



7.6.1 Cromdale Grove (Fig 7.2)

CLS, ORM and TOR were the most efficient at removing Cd (100 – 125g), followed closely by TRI with just under 100g of Cd removed. Compared to other taxa, TOR and TRI both contain a significantly larger proportion of Cd in their leaves. TRI accumulated the largest quantities of Zn (over 14kg), but 75% of this was located in foliage (Table 7.4). CLS and GER both accumulated over 10kg of zinc, although large proportions were also located in foliage. Although total zinc accumulation was reduced in ORM to 8kg, almost 6kg was located in the stem and a similar trait could be observed in Cd and Cu uptake of this taxa. GER was able to remove most Cu and Ni into the stem. A large amount of Ni uptake for all species was into the foliage.

7.6.2 Merton Bank 3 (Fig 7.3)

CAL was able to accumulate increased concentrations of Cd (25g total, 20g in stem) and high Zn (2000g), reflecting an overall lower concentration of these elements in the soil, and therefore lower uptake. Zinc uptake was, similarly to other sites, dominated by TRI but still with a 75% proportion in the leaves, a pattern that was repeated in Cu uptake for this taxa. Copper uptake was most pronounced in CLS, with 90% of the 200g total located in the stem. ALN also made a significant impact by accumulating 160g Cu, in the stem, although no foliar data was extrapolated for this species. Although seemingly insignificant, LAR was able to remove 50g of Cu into the stem on limited biomass due to poor survival. Nickel and lead concentrations are very low and dominated by foliage uptake. Arsenic removal is very low for all species, although it was not possible to quantify and extrapolate LAR foliage, which had the highest concentrations of As (see Chapter 5).

7.6.3 Fazakerley 2 (Fig 7.4)

CAL accumulated significantly more Cd than any other species or taxa, with approximately 580g in total of which 420g was in the stem. All *Salix* taxa showed excellent removal of Cd on this site, predominately into stem tissue. A similar pattern was repeated with Zn, and CAL followed by TOR being the most effective at uptake. Both *Populus* taxa exhibited the lowest concentrations of Cd, Zn and Cu uptake, possibly reflecting their general poor growth on this site. Similar assumptions could be made for ALN, no longer exhibiting comparatively high Cu uptake as on previous sites. CLS and TOR were the greatest accumulators of copper, and all *Salix* taxa showed 80-90% of Cu located in the stem GER was the only variety to accumulate possibly significant amounts of Ni into the stem, although very low.

7.7 Metal Uptake Partitioning

If an alternative strategy of harvesting after leaf fall in Year 2 is adopted (Tables 7.4 – 7.6), it is possible to examine the percentage of the total amount of metals that will be lost due to leaf senescence. Nickel and Lead have been omitted due to unrealistic targets for extraction processes.

Table 7.4. Extrapolated uptake figures for Cromdale Grove 1 (CRM1) showing total metal uptake after 2 years (F1+F2+S2) and percentage of this amount lost in Year 1 foliage (F1) and Year 1 + Year 2 foliage (F1+F2).

	Cd			Cu			Zn		
	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2
CAL	73	15	33	248	14	78	8866	23	58
CLS	140	9	39	547	17	67	13755	16	51
GER	94	6	24	692	10	56	12034	10	51
GHY	47	22	63	282	28	58	6755	25	84
ORM	120	16	38	612	18	79	10817	21	44
TOR	114	10	38	550	14	86	10709	21	66
TRI	93	0	69	363	31	78	16209	8	71

Table 7.5. Extrapolated uptake figures for Merton Bank 3 showing total metal uptake after 2 years (F1+F2+S2) and percentage of this amount lost in Year 1 foliage (F1) and Year 1 + Year 2 foliage (F1+F2).

	Cd			Cu			Zn		
	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2
CAL	28	9	30	109	6	22	2232	19	55
CLS	14	28	52	235	17	31	1847	40	74
GER	13	13	34	114	15	30	1755	21	55
GHY	18	26	57	195	24	47	2785	34	76
ORM	2	100	100	21	100	100	703	100	100
TOR	10	30	100	154	16	34	2135	23	64
TRI	21	8	42	252	28	70	3492	11	75

Table 7.6. Extrapolated uptake figures for Fazakerley 2 (FAZ2) showing total metal uptake after 2 years (F1+F2+S2) and percentage of this amount lost in Year 1 foliage (F1) and Year 1 + Year 2 foliage (F1+F2).

	Cd			Cu			Zn		
	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2	Total Uptake g / ha / 2yr	% lost F1	% lost F1+F2
CAL	635	11	31	311	6	20	12292	23	50
CLS	458	12	38	531	10	23	7297	18	52
GER	331	18	43	331	10	18	6874	22	55
GHY	129	27	50	225	21	45	3634	28	67
ORM	282	13	37	315	17	34	6606	16	54
TOR	394	10	35	415	12	23	7955	7	45
TRI	220	22	56	178	34	70	5804	23	77

7.8 Long term models

By using the longer term equation, 10(F2 + S2) + CSt + Rt (Equation 7.2), we can make a prediction for the total metal removed by a crop over its lifespan, in this case a period of 20 years. This is shown in Tables 7.7 – 7.9 for the same taxa and sites as described previously in this chapter. Year 0 cutback stem total metal concentrations (CSt) and root bole total metal concentrations (Rt) are omitted from these calculations as no data was collected for these parameters in the present study.

Table 7.7. Total net metal removal for Cromdale Grove 1 (CRM1), 10(F2 + S2) over a 20 year crop lifespan (kg ha^{-1})

	Cd	Cu	Ni	Pb	Zn
Species Mix	0.62	2.78	0.70	0.28	67.40
CAL	0.62	2.14	0.60	0.16	68.23
CLS	1.28	4.55	0.93	0.45	115.64
GER	0.88	6.24	1.26	0.45	108.68
GHY	0.37	2.04	1.20	0.39	50.83
ORM	1.01	5.02	0.72	0.28	85.13
TOR	1.03	4.76	0.99	0.35	84.86
TRI	0.92	2.52	1.27	0.70	149.66

Table 7.8. Total net metal removal for Merton Bank 3 (MER3), 10(F2 + S2) over a 20 year crop lifespan (kg ha^{-1})

	Cd	Cu	Ni	Pb	Zn
Species Mix	0.09	1.09	0.15	0.10	11.51
CAL	0.25	1.03	0.21	0.12	18.00
CLS	0.10	1.96	0.19	0.12	11.05
GER	0.11	0.97	0.16	0.15	13.85
GHY	0.14	1.48	0.29	0.22	18.37
ORM	0.00	0.00	0.00	0.00	0.00
TOR	0.07	1.30	0.12	0.11	16.41
TRI	0.19	1.82	0.39	0.26	31.07

Table 7.9. Total net metal removal for Fazakerley 2 (FAZ2), 10(F2 + S2) over a 20 year crop lifespan (kg ha⁻¹)

	Cd	Cu	Ni	Pb	Zn
Species Mix	2.02	2.03	0.34	0.26	40.46
CAL	5.62	2.91	0.10	0.77	94.41
CLS	4.01	4.79	0.38	0.53	59.88
GER	2.70	2.97	1.32	0.25	53.66
GHY	0.94	1.78	0.43	0.18	26.26
ORM	2.47	2.61	0.35	0.18	55.61
TOR	3.53	3.65	0.40	0.26	73.65
TRI	1.73	1.18	0.25	0.39	44.50

The values in Tables 7.7 – 7.9 rely heavily on the assumptions listed at the start of this chapter, and therefore may contain errors that will exacerbated over time. If stem only harvesting were adopted then the figures would be reduced by the percentages shown in Tables 7.4 – 7.6. However, it may be that these are conservative figures, as biomass yields usually increase with subsequent harvests given sufficient site conditions (Chapter 6). In addition the values calculated are for stem and foliar uptake at time of harvest only and do not include root or stem cutback uptake, which are discussed in more detail later in this chapter.

7.9 Metal removal rates

Based on the yields and uptake figures above it is possible to calculate the time it would take to reduce the contamination levels in a predefined volume or depth of soil. These calculations include the additional assumptions of a constant bulk density of 1 and use of an average soil concentration figure for each plot, as well as the assumptions listed in Table 7.1. Figures 7.5 – 7.7 show metal removal rates for the 3 example plots examined previously in this chapter.

Figure 7.5. Prediction of metal removal from top 10cm and top 30cm of soil on Cromdale Grove 1. Dashed vertical bars indicate likely lifetime of a crop (20-30years) and concentrations on the right hand axis represent actual soil metal concentration onsite.

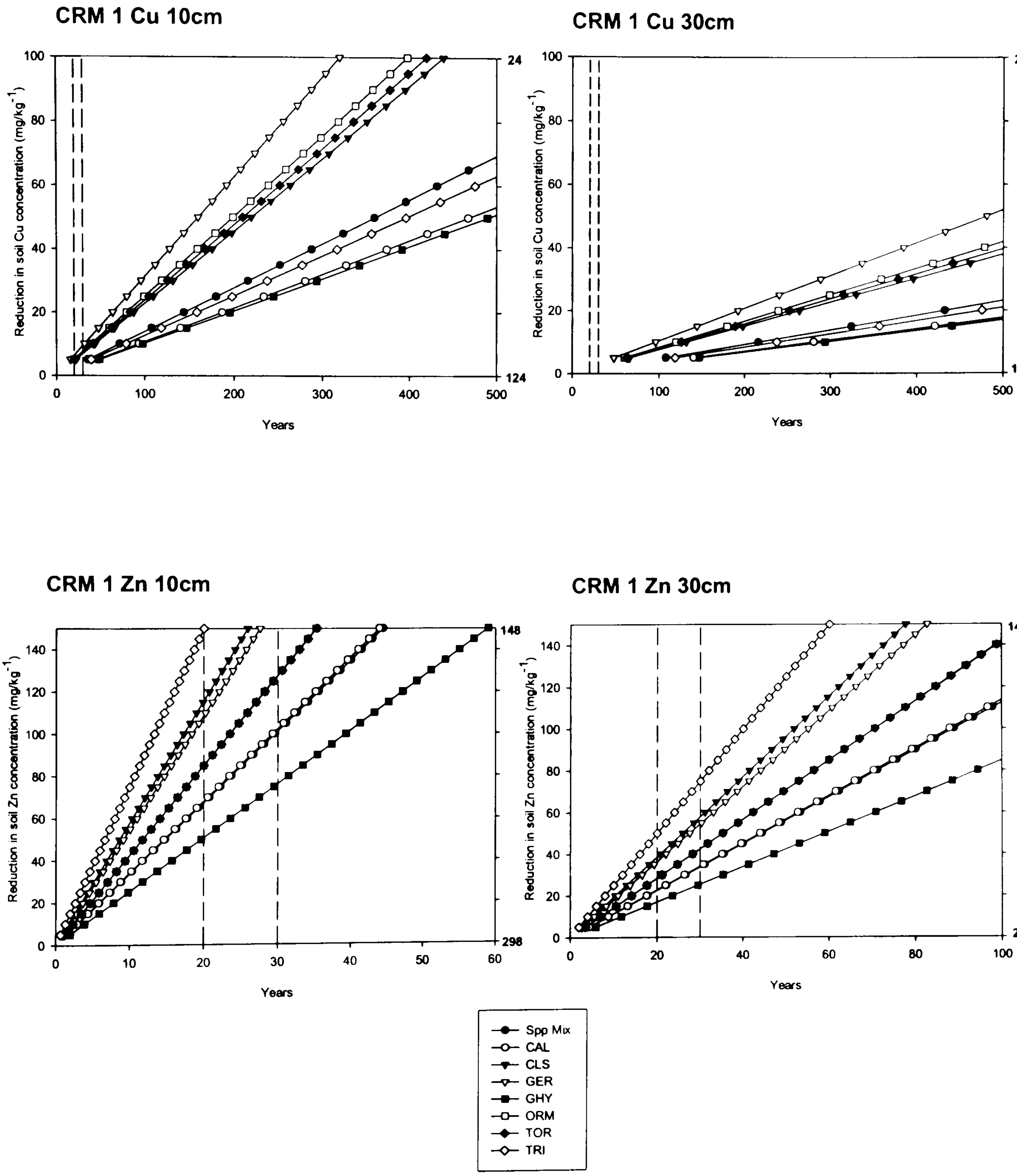


Figure 7.6. Prediction of metal removal from top 10cm and top 30cm of soil on Merton Bank 3 vertical bars indicate likely lifetime of a crop (20-30years) and concentrations on the right hand axis represent actual soil metal concentration onsite.

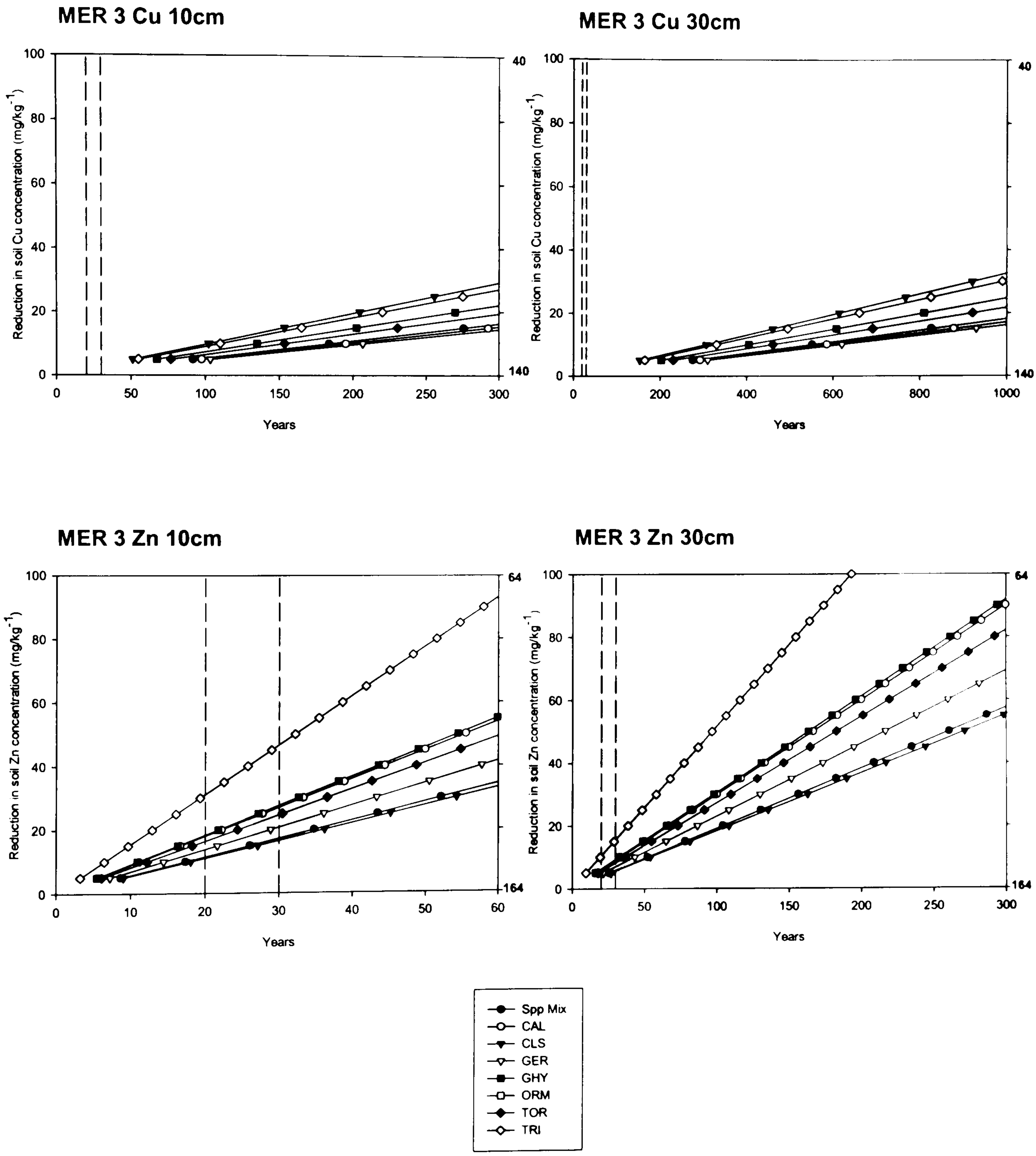


Figure 7.7. Prediction of metal removal from top 10cm and top 30cm of soil on Fazakerley 2. Dashed vertical bars indicate likely lifetime of a crop (20-30years) and concentrations on the right hand axis represent actual soil metal concentration onsite.

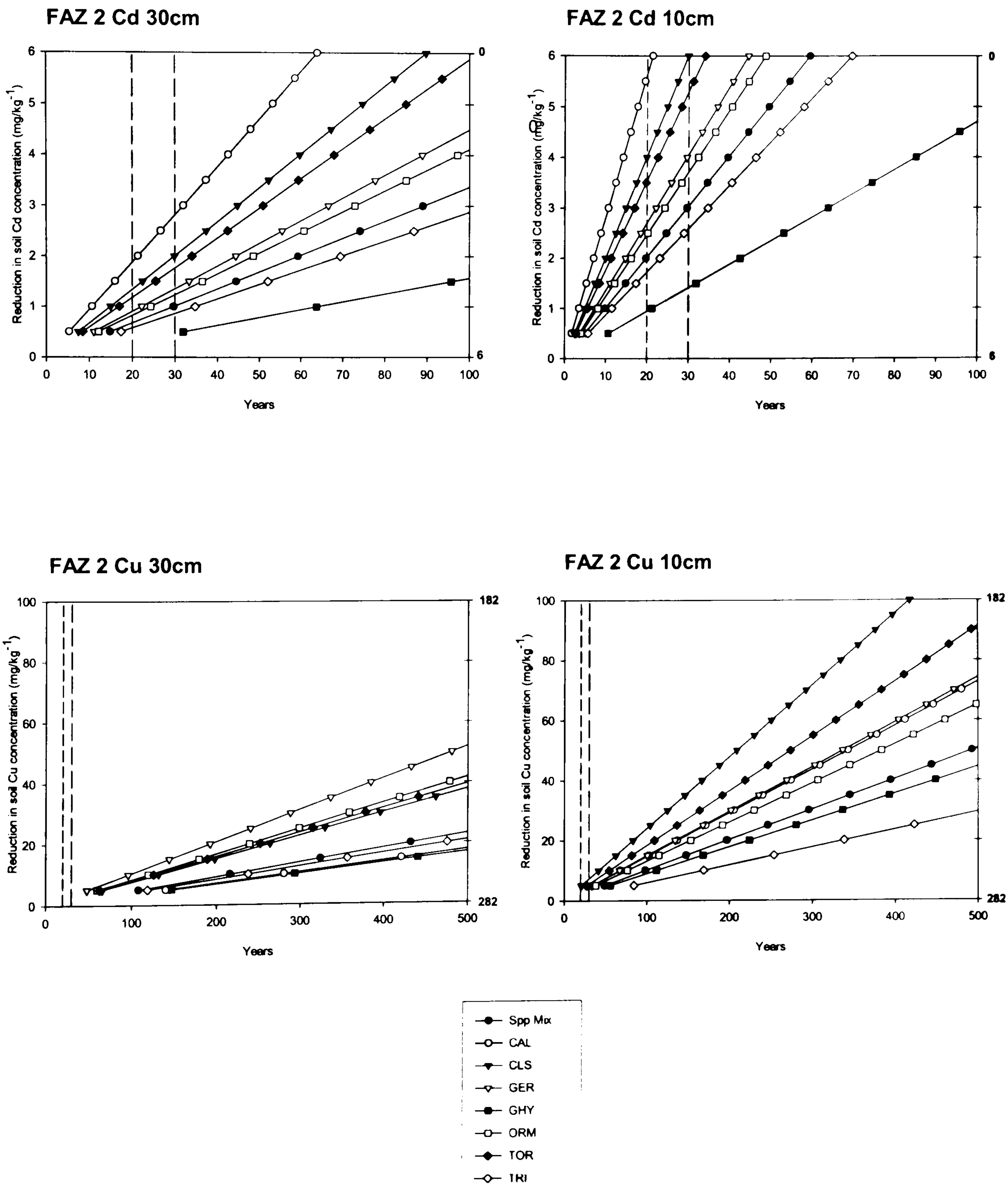
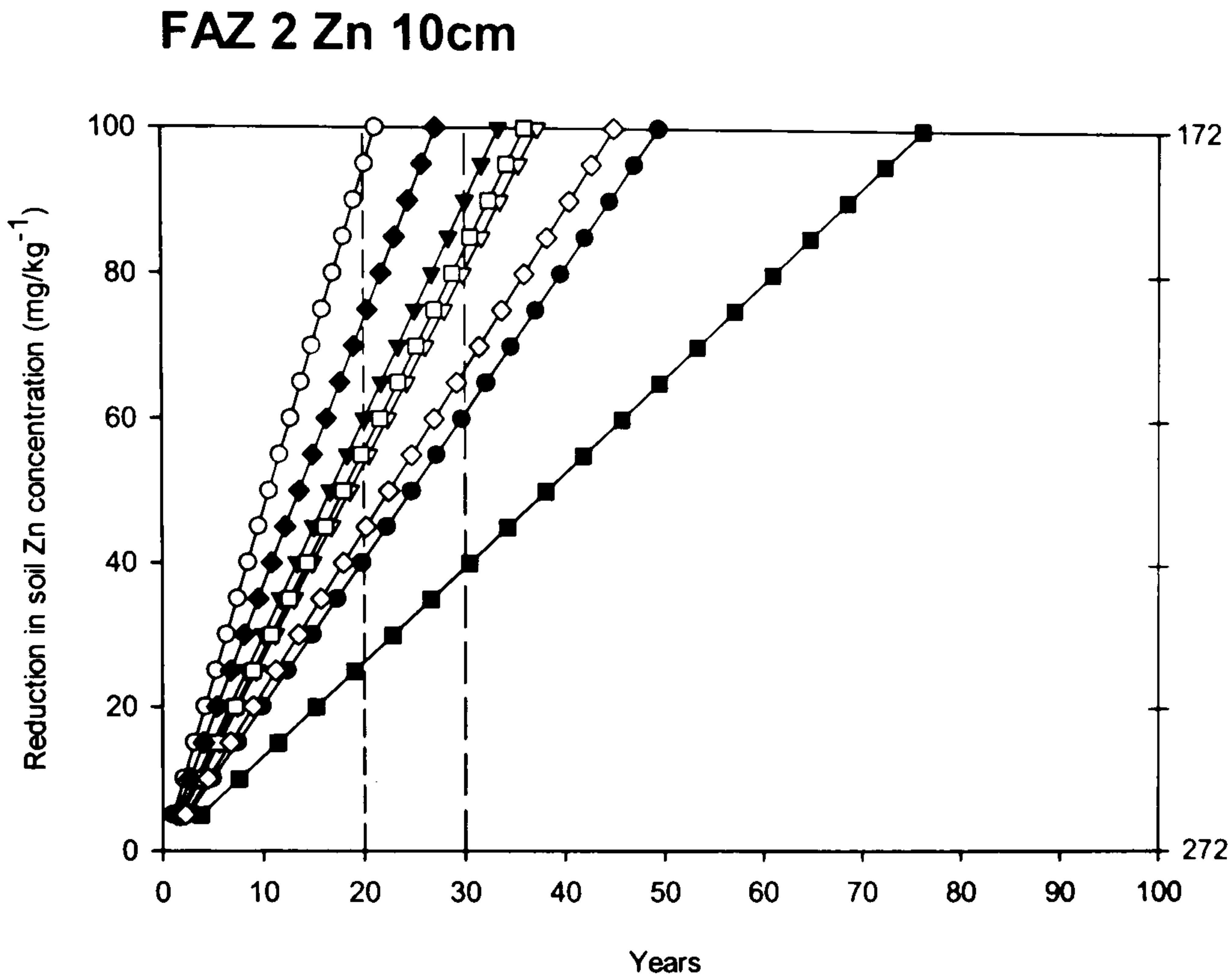
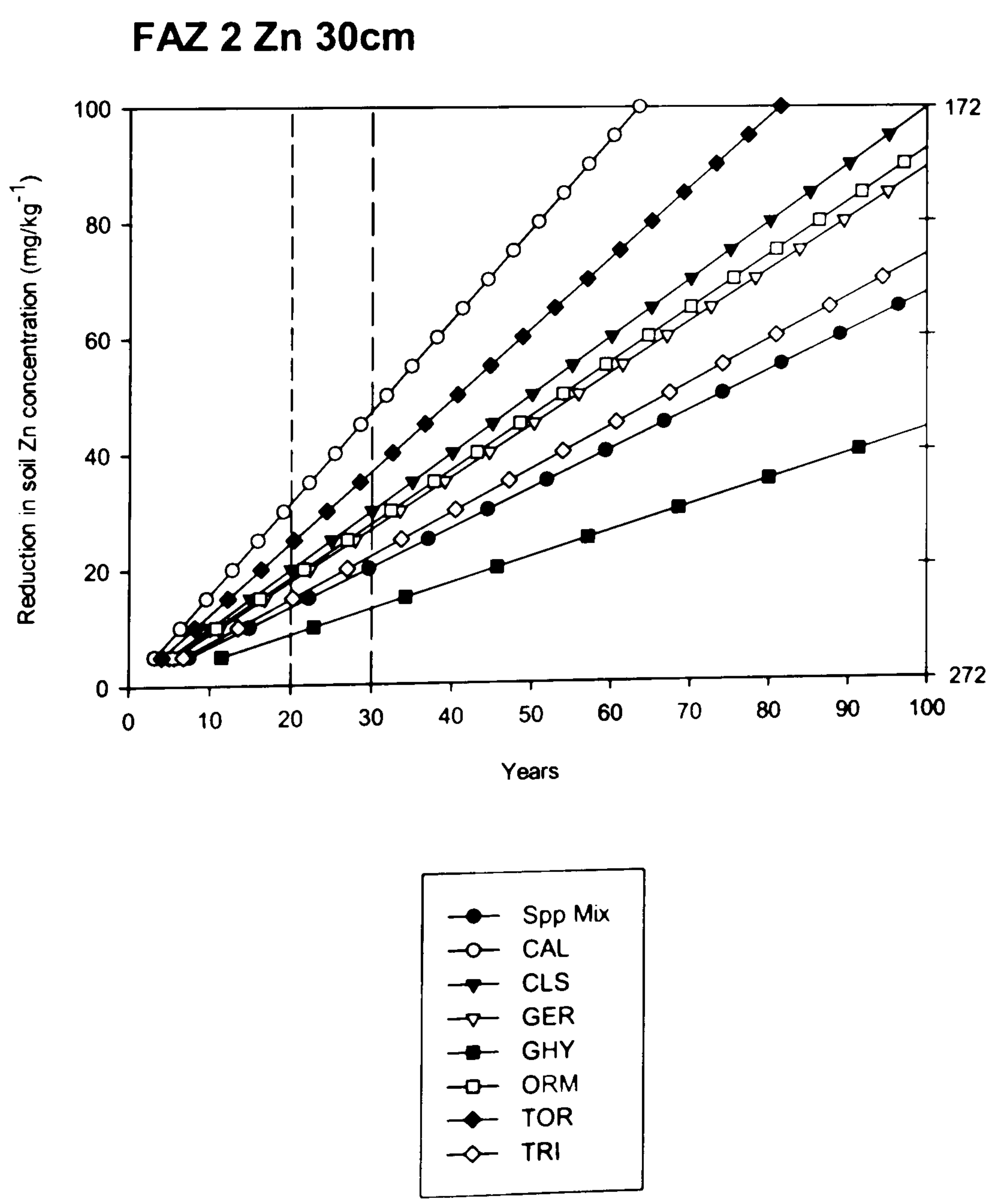


Figure 7.7 (cont'd) Prediction of metal removal from top 10cm and top 30cm of soil on Cromdale Grove 1. Dashed vertical bars indicate likely lifetime of a crop (20-30years) and concentrations on the right hand axis represent actual soil metal concentration onsite.





Calculations are based on total soil metal reductions (as opposed to EDTA or $CaCl_2$ extractable), as total extractions are currently used in practical risk assessment and statutory guidance. These figures are useful to consider the possible time taken to remediate an area of contaminated land to suitable soil concentrations.

Copper removal was slow on all sites, and well outside any realistic timeframe for clean up, although rates are increased at higher concentrations such as FAZ 2, however this still translates into 100 years for a 20ppm reduction.

Zinc removal was substantial, especially at high soil concentrations. However, at lower soil concentrations, removal slows suggesting that removal may slow gradually as soil concentrations fall. TRI exhibited the quickest clean up rate on MER and CRM, taking just 20 years to remove 150ppm Zn from 10cm soil on CRM and 30ppm in the same time period on MER 3 where Zn soil concentrations were almost half. However, much of the Zn in TRI was contained within the foliage (71-77%, Tables 7.4 to 7.6) and caution should be adopted in these extraction figures unless foliage can be harvested intact. Other taxa showed high accumulation rates, CAL and TOR can reduce Zn by 100pm in 20-25 years on FAZ 2 within the top 10cm of soil.

Concentrations of Cd can be reduced quickly, and changes in just a few ppm can alter the contamination status of the land dramatically (Chapter 4). For example, a reduction of just 3ppm on FAZ 2 for Cd in the top 10cm could remove the site from a statutory definition of being contaminated. Figure 7.6 shows this could be possible in just 10 years. Cadmium was only examined at FAZ 2 as other plots showed very low Cd soil concentrations. On FAZ 2 the average soil concentration was 6mg kg^{-1}. According to the extrapolations CAL and CLS can remove all soil Cd from the top 10cm in the 20-30 year lifespan of the crop. A larger, more realistic depth of soil using the same taxa could reduce the Cd content of the soil in the top 30cm by 2-3ppm over the same 20-30 year timeframe.

7.9.1 Root biomass and other uptake mechanisms

No empirical measurements of root biomass or root metal concentration were carried out in the present study. Root uptake of metals is known to be a major sink in relation to metal extraction and mobility. Although roots cannot be harvested on a cyclical basis they may collect large quantities of metals within their tissues, rhizosphere or adjacent soil over the lifetime of a crop. Roots can account for a large biomass proportion of a functioning coppice system, Rytter (1999) proposed that the below ground portion can contribute up to 50% of annual net primary productivity in *Salix*.

Studies have attempted to assess root biomass of *Salix* and *Populus*, but little firm data exist, favoring assessments of rooting habits such as depth and root type. Crow and Houston (2004) found an average of 80% (range 75% - 95%) of *Salix* and *Populus* roots counted were in the top 36cm of soil, corresponding with the ploughed Ap soil horizon. Furthermore 80% of these were fine roots of less than 2mm diameter. Rytter (1997) reported similar findings, 90% of root biomass in 4 year *Salix* stools occurred in the top 30cm of soil, however, maximum rooting depth can stretch from 1.5m in 2 year old poplars to 5m in 4 year old trees (Friend *et al.* 1991).

Root:Shoot ratios change quickly with time given the constantly fluctuating biomass of the above ground parts of the tree, and are considerably larger in the early years after planting or coppicing in *Salix* (Volk *et al.* 2001). Spacing of plants also affects root biomass, at denser spacing individual *Salix* plant root biomass is greatly reduced, although on a per area basis the results are very similar, between 3.72 - 4.71 Mg ha^{-1} (Volk *et al.* 2001).

The large proportion of fine root systems allows for maximum rhizosphere interaction and may help to accumulate increased metal quantities. Given that roots account for such a large proportion of total biomass, it may be an essential prerequisite, or at the least prudent to remove the root bole at the end of the lifespan of the crop. This would maximize the metals extracted and prevent re-mobilisation of metals into the environment if root material is simply ploughed or milled and allowed to decompose in-situ. Removal of the root bole would be a relatively simple operation and would be necessary if there was a move to an alternative land use.

Additional biomass and metals will be removed in the Year 0 cutbacks, although this would seem to be relatively insignificant. Biomass harvested at Year 0 on CRM 1 would only account for 4 – 6.5% of biomass harvested per hectare per year after 2 full growth seasons

7.10 Disposal Technologies

If phytoextraction is to be successful then careful consideration must be given to the processing and ultimately disposal of plant material that may contain high concentrations of toxic metals. This critical step in a successful remediation scheme is frequently overlooked. Garbisu and Alkorta (2001), Mulligan *et al.* (2001) and Sas-Nowosielska *et al.* (2004) all review specific disposal technologies and examine the current technology status, the key points of which are summarised below.

Sas-Nowosielska *et al.* (2004) describes a two-step process for harvested material containing metals; initially a bulk reduction phase followed by a disposal phase. Bulk reduction pretreatment to reduce crop volume and water content is essential (Blaylock and Huang 1999), improving technical parameters of the material and lowering transportation costs for off site processing. Composting may reduce biomass by 25% in the case of forbs and grasses (Hetland *et al.* 2001), although it was found the process may enhance solubility of metals (in this case Pb). Plant uptake systems that have used assisted extraction using EDTA or other chelates may produce very mobile metal concentrations in composting (Sarret *et al.* 2001). Those without added chemical extraction have shown that zinc within foliage is present in water soluble forms (Zhao *et al.* 2000), requiring careful containment of materials and leachate if composting is to be used. In *Salix* and *Populus* a large proportion of the material is woody biomass and will not compost quickly, although large reductions in water content will be possible and composting of leaves is likely to reduce volumes.

Compacting is another pretreatment option (Blaylock and Huang 1999) and has similar advantages as for composting although the process should be quicker, however there is little field data on this process.

Pyrolysis decomposes material under anaerobic conditions at moderate temperatures, forming pyrolytic gas or liquid oil (in fast pyrolysis) and coke or char (Bridgwater *et al.* 1999). The gas or oil can be used as a fuel and the coke, containing metals in a reduced volume for secondary disposal. Recent work by Koppolu *et al.* (2003) shows that 98.5% of metals are retained in the char and there are no emissions to air as the process is entirely hemetic. Final disposal can be carried out by incineration, smelting or ashing. Smelting is feasible but requires a suitable nearby plant.

Ashing has received most attention, Hetland *et al.* (2001) co-fired lead contaminated biomass with coal, reducing the biomass by 90% and partitioning lead into the ash. Systems have tentatively been suggested to recover metals from the ash (Garbisu and Alkorta 2001). Although more data on combustion systems and ash recycling is needed, recent EU studies such as BIORENEW (Riddell-Black 2002) have examined these topics. Metals such as Cd, Pb and Zn can be recovered in fly ash, reducing their concentration in bottom ash which can then be applied to future crops as a fertilizer, creating a sustainable system (Riddell-Black 2002), although As and Cu concentrations in bottom ash remain high. Using contaminated biomass sources is therefore possible, but requires careful consideration and management. If used in co-combustion with other dirty fuels such as coal or municipal solid waste (MSW) the metal loading in biomass is unlikely to cause a problem. Technologies such as flue gas cleaning are in place to capture contaminants from dirty fuels and ashes from such facilities, usually high in metals, are disposed in controlled practices. Current UK policy in conventionally fired power stations promotes co-combustion of other fuel sources, including biomass (ENDS 2003). If used as a sole fuel source however consideration must be given to appropriate cleaning and filtering technologies at the plant (Riddell-Black 2002). Other disposal options include direct disposal at a hazardous waste landfill or other disposal facility, which is likely to be as expensive and unsustainable.

Recent lab scale experiments of liquid extraction techniques using chelating agents have shown to remove 98.5% of Pb from biomass using liquid chelators (Hetland *et al.* 2001), although a production scale model has yet to be demonstrated (Mulligan *et al.* 2001).

Removing metals in woody biomass may have advantages over other plant material. Woody materials will not leach metals easily and are easier to handle although will represent higher volumes for disposal. Foliage with high metal content may be a problem and requires suitable containment if composted.

After the failure of the UK's largest commercial biomass power station, domestic interest in energy crops diminished in the late 1990's. However, a recent government policy change now places biomass power firmly up the agenda (DTI 2004) with ambitious targets of between 9-12% of the UK electricity supply from biomass by 2050 (ENDS 2004). A report as part of a recent DTI review ascertained that crop yields need to improve by 30% to be commercially viable (i.e. with no subsidy). Many growers of energy crops believe this is achievable. A sustainable system based on series of 2MW local power generation facilities requiring about 1,000ha of crops each per year could be feasible. In addition the report emphasizes that energy crops can only be economically viable on set aside land compared to other traditional agricultural crop returns (LEK Consulting 2004). Brownfield and derelict land can also be classed as set-aside, although may require additional cultivation.

Co-firing of biomass and other non fossil fuel products with traditional coal is becoming more common, under national obligations for renewable energy and international obligations for CO_2 emissions reduction (DTI 2003). On a local scale the main coal fired power station in the Merseyside area has been co-combusting 10% non fossil fuels for some time and is seeking to expand this operation (ENDS 2003).

7.11 Conclusions

There are many factors that differ within individual plots, such as soil depth, pH, fertility and metal concentration that will ultimately have an effect on the rate of biomass growth and the amount of metal translocated to above ground portions of the tree. Earlier chapters have demonstrated that these variables can change rapidly within a small area (Chapter 4). For these reasons it is not possible to select a species or taxa that consistently achieves high uptake rates across all plots. Although long term data are not yet available it may be possible to use field trials and methods similar to those used in this experiment to test a variety of species in-situ with a view to a wider planting and remediation plan. Given optimum planting and harvesting times it may be possible to assess uptake, growth and survival rates for species within 2.5 years, before committing to full scale planting.

Earlier harvesting to capture foliage would be technically possible, although no field data are yet available to support this. Harvesting would have to be carried out using a stick harvester and not an *in-situ* chipper, as leaves would block this device (Ian Tubby, *pers. comm*). Suitable containment methods would be required to prevent leaf dispersal into the wider environment, especially if using trees such as *Populus* TRI where between 70-80% of Zn or Cu removal may be located in the foliage. If this cannot be achieved, or if the area is particularly sensitive to ecological metal input, then species that retain large amounts of metals in the stem that cannot be readily re-dispersed into the environment should be selected.

Volumes of leaf material could be reduced via composting if suitable collection systems were in place to capture contaminated leachate. This process could also be used to remove water from the stems prior to final disposal. Recent UK policy suggests there is a strong argument for co-combusting biomass that may be contaminated with traditional dirty fuels, thereby contributing to renewable energy targets and allowing a safe disposal system. It would seem there is a demand for such a product if the economic viability can be achieved. This may be possible with increases in planting density to increase yield. This experiment was planted at a rate of approximately 13,333 plants per ha, to satisfy experimental design requirements. Planting densities of similar species grown commercially are now possible at 111,000 plants per ha to achieve maximum yield benefits, although rates of between 15,000 and 20,000 would seem to be the most beneficial with economic considerations (Bullard *et al.* 2002).

The interaction of metals with tree roots is a key element that has not been addressed in this experiment and should be investigated in future work.

The most noticeable overall trend is that trees are able to remove substantial and significant quantities of Cd and Zn. Cd removal would seem feasible in an acceptable timeframe, for low value brownfield land. This corroborates other experiments where timescales such as 33 years (Vervaeke *et al.* 2003) have been proposed in order to reduce Cd to acceptable concentrations. Cu extraction is slow, reflecting a higher Zn uptake and exclusion of Cu and also low mobility of Cu in plants irrespective of source (Nissen and Lepp 1997).

Chapter 8: Conclusions

The Community Forest Initiative has pioneered new approaches to managing land in and around urban areas, with half the population of England living within easy reach of a Community Forest (The Countryside Agency 2003). During the first 15 years of this programme, a large proportion of the expansion of a wooded landscape has been directed towards derelict, underused and neglected land, especially in The Mersey Forest region. Regeneration via reforestation of brownfield and potentially contaminated land has become central to improving the community forest resource and contributing to successful economic regeneration of run down areas (NWDA 2003). At the same time drivers for the expansion of biomass crops for renewable energy and carbon sequestration have also emerged (Cannell 2003; DTI 2004). New UK statutory guidance on contaminated land has focused attention on contaminated land assessment and management; the use of trees for phytoremediation has shown promise in this area. All these factors combined suggest the need for better understanding to address contamination issues associated with planting trees on brownfield land.

Phytoremediation has been extensively promoted as a reclamation tool but previously there has been limited field demonstration on brownfield land with low-level and varied contamination histories. Many of these sites are within urban landscapes and require attention to manage contamination risk, regenerate or utilise the area as a community resource. Investigations into the nature of brownfield sites and their interactions with trees (e.g. detailed soil contaminant mapping, consideration of appropriate guidelines, yield and uptake measurements) have been inadequate. Those that do exist have not been carried out in a real-time, field-scale and multi-site environments.

In the present study, the importance of detailed site investigations was demonstrated. Considerable variation of contaminant type and concentration was typical between sites but also significant within sites. A heterogeneous dispersion of metals, with clearly defined hotspots, was found on most sites in the present study. Brownfield land probably differs in this respect to many sites where previous studies have been carried out, for example on sludge-amended agricultural land. The identification of hotspots allows targeted assessment and remediation of soil contamination.

Contaminated land guidance is problematic and inadequate in the context of tree planting and community forestry. Previous guidance, such as ICRCL (formally withdrawn since 2002), provides a conservative indication of phytotoxicity (Dickinson *et al.* 2000). Currently, there is no practical guidance available for phytotoxicity threshold concentrations. Assessment of contaminated land is driven by risk based guidance (CLEA) that considers primarily risks to human health and controlled waters. Within the current CLEA framework there is no amenity land use defined in the risk assessment process, although research is underway to better define an amenity exposure scenario. Therefore conservative soil guideline values (SGVs) based on residential uses have to be applied where public access is required. The current study shows this will not always be appropriate using the one size fits all methodology currently available. Trees are unlikely to increase risks on heavy metal contaminated land and could be used to break source, pathway, receptor contaminant linkages, via dense planting on contaminated hotspots to stop ingestion or contact with soils, or to stabilize exposed substrates.

Risk assessment is a complex and continually developing issue. In an increasingly litigious society the onus will be on the site developer to prove that no risks to human health are present for community forestry schemes. Further research into the relationship between people, contaminated land and tree planting is required, via site specific models utilizing data on metal mobility within soil and plants that has been gained from this study

Key goals must be addressed and considered early in the design and planning of brownfield remediation. In the context of community forestry, there is an opportunity (i) to use trees to stabilise contamination (for example of As and Pb) or (ii) to use *Salix* to extract metal and reduce soil metal concentration (particularly of Cd and Zn).

All *Salix* taxa used in the present study accumulated a range of heavy metals in differing quantities, with Cd and Zn uptake most significant and broadly correlated with soil concentrations. Other studies have reached similar conclusions (Pulford *et al.* 2002; Vandecasteele *et al.* 2002; Klang-Westin and Eriksson 2003). Cd and Zn were accumulated to significant concentrations in above ground biomass, even when there were very low levels present in the soil. CAL and GER exhibited the greatest concentration factors for Cd, approximately 7-9 times soil EDTA concentration could be accumulated in the stem and 9-13 times in foliage from soils with very low concentrations of Cd. Pb was not absorbed into the aerial parts of trees despite elevated soil concentrations. Larch was able to accumulate Arsenic at ten times the rate of other species, but not at significant concentrations for phytoextraction.

Significant relationships were identified between stem and foliar concentrations of Cd and Zn, illustrating the mobility of these metals within trees. Correlations were found between Cd in *Salix* stem and foliage with r^2 values between 0.92 – 0.98 in individual taxa and r^2 of 0.88 when data on all *Salix* taxa was pooled. If consistent, these results could have implications for non destructive measurement of stem metal concentrations by using foliar values to predict stem concentrations.

Similar patterns were evident when correlating soil metal concentrations with stem metal concentrations for Cd and Zn. EDTA and total soil extractions of Cd and Zn showed some relationships with stem concentrations, which were more pronounced for Cd. In this case a relationship between soil Cd concentration and stem Cd concentration was found for a range of sites. This too may allow future non-destructive predictions of metal uptake based on soil concentrations.

Non coppice species used such as Alder, Birch and Larch may have some potential for phytoremediation and biomass production schemes. *Alnus* and *Larix* both accumulated Cu at greater quantities than some *Salix* taxa, with an average stem concentration of 20 mg kg^{-1} and 55 mg kg^{-1} respectively. Growth of birch and larch were negatively impacted by competition from surrounding *Salix* and *Populus* species that grew considerably faster, however *Alnus* competed effectively, producing elevated biomass yields. Utilising non coppice species such as these may have considerable advantages for biodiversity of SRC stands and protection of *Salix* from rusts and other diseases able to thrive in monoclonal environments. The penalty paid for low biomass of birch and larch may be offset by prevention of *Salix* mortality due to disease.

Planting trees did not increase in EDTA-extractable (bioavailable) metals in soil after three growing seasons. This has considerable relevance to risk management modeling. In some situations there was evidence that soil metal concentrations were actually reduced during the period of study. Whilst both EDTA and $CaCl_2$ soil extractants were used, EDTA proved a more useful extractant for a wider range of metals. $CaCl_2$ may more accurately represent actual soil conditions, but at low soil metal concentrations frequently encountered on brownfield sites, recoveries were too low to be detected by routine laboratory techniques, thus proving difficult to generate meaningful quantitative results for comparison. More accurate techniques are being proposed to examine soil bioavailable metals, such as DGT (Song *et al.* 2004), but the use of EDTA was an efficient and valid representation of different contaminants when dealing with large numbers of samples from varied sites.

Attention to correct field preparation, planting and maintenance on brownfield and contaminated sites resulted in an average of only 10% mortality for most coppice species in the establishment year, rising to little over 20% after 3 growing seasons. Vandalism was not a serious problem at most sites. Willows reacted well to coppicing, but poplar did not, suggesting better management may be to leave them longer before cutting back in this part of the UK. Biomass yields of most willow species were comparable to those grown on agricultural land and several sites produced yields above the economic threshold for commercial cultivation of 10 odt ha^{-1} yr^{-1}. Intensive site preparation is required for short-rotation coppice, but this is less demanding than for agricultural crops and is demonstrated in the present study to be feasible with appropriate aftercare. Brownfield sites are frequently highly fertile, but fertilisation and irrigation may be required to maintain high yields over a full 20 – 25 year lifecycle of an SRC crop. Biomass production varied with site and species, but average yields from the best performing willows were up to 13 odt ha^{-1} yr^{-1}, making commercial profitability a serious consideration on 50% of the sites studied. Willows yielded more then Poplar; but Alder biomass production also proved significant, producing a maximum of 8 odt ha^{-1} yr^{-1}, the same as the best performing poplar, TRI.

Overall, biomass yields were favorable, but it is uncertain if they can be sustained over the lifetime of a crop due to soil nutrient depletion. The use of fertilizers or other amendments could be used to increase yield further and may be required later in the crop lifecycle to maintain production. In the present study sewage cake, correctly applied, produced an increase in yield of 70-80 % in some SRC species over non amended soils. Conversely incorrectly applied amendments may reduce yields due to loss of soil structure and watterlogging.

The *Salix* taxa CAL and GER exhibited lower biomass yields than other *Salix* taxa, but also showed lower mortality and high concentration factors. Other taxa with increased yields and lower concentration factors were able to accumulate similar quantities of Zn and Cd on sites when considered using a mass balance model. This study reinforces findings of Pulford *et al.* (2002) that it is crucial to consider not only innate metal uptake ability, but also biomass yield and species mortality to generate an accurate assessment of metal removal in the long term.

Metal uptake into stem and foliage, biomass yield and species mortality field data collected over three growing seasons has not previously been available for brownfield sites. Collation and extrapolation of these data suggest that enough Cd and Zn can be removed to make phytoextraction feasible in the longer term. These metals could be removed in substantial quantities to significantly lower concentrations of metals in hotspots. This would require (i) continued uptake of metals into plant tissues over a longer period of years, (ii) harvest of plant in late summer prior to leaf fall. Significant metal uptake can be lost through foliage, especially for Zn with over 50% lost in 2 years if leaves are allowed to fall, especially in some *Populus* species due to their increased leaf biomass. Additional removal of metals through harvest of the root bole on completion of the remediation process would probably contribute to further reduction.

Further extrapolation of field data can be used to predict total metal removal over the lifetime of a crop, in this case 20 to 30 years. The best site / species combinations could theoretically accumulate 5.5kg of Cd and 150kg Zn per hectare over 20 years, translating to a reduction of 6ppm Cd and 150ppm Zn in soil. The Cd removal is potentially more significant as changes of just 1ppm in soil concentrations can alter the statutory designation of contamination.

Tree planting is a useful long term remediation tool on brownfield land contaminated with low, but elevated soil concentrations. This study has demonstrated that it is feasible to grow trees on brownfield, contaminated sites for a range of end uses, including community forestry, biomass production and phytoremediation.

Chapter 9: References

Adegbidi, H. G., Briggs, R. D., Volk, T. A., White, E. H. and Abrahamson, L. P. (2003). Effect of organic amendments and slow-release nitrogen fertilizer on willow biomass production and soil chemical characteristics. *Biomass and Bioenergy* **25**: 389-398.

Alker, G. R., Riddell-Black, D., Smith, S. R. and Butler, D. (1997.). Investigations of nutrient uptake by willows in a soil-less system. *Aspects of Applied Biology*. **49.**: 173 -181.

Allen, S. E. (1989). Chemical analysis of ecological materials. Oxford, Blackwell.

Allot and Lomax (1997). The assessment of closed landfill sites for community forest use. Stage 1: Desk study and preliminary site investigation and works. Volume 3: Ecological assessment. Unpublished report to the Mersey and Red Rose Forests.

Alloway, B. J. (1995a). Cadmium. In: Heavy Metals in Soil. Alloway, B. J.(Eds.) Glasgow, Blackie.

Alloway, B. J. (1995b). Heavy metals in soils. London, Blackie.

Alloway, B. J. and Jackson, A. P. (1991). The Behaviour of Heavy Metals in Sewage Sludge-Amended Soils. *The Science of the Total Environment.* **100**: 151-176.

Alriksson, A. and Eriksson, H. M. (2001). Distribution of Cd, Ck, Pb and Zn in Soil and Vegetation Compartments in Stands of Five Boreal Tree Species in N.E. Sweden. *Water, Air and Soil Pollution: Focus* **1**: 461-475

Anderson, C. W. N., Brooks, R. R., Chiarucci, A., LaCoste, C. J., Leblanc, M., Robinson, B. H., Simcock, R. and Stewart, R. B. (1999). Phytomining for nickel, thallium and gold. *Journal of Geochemical Exploration* **67**: 407-415.

Andronikov, S. V., Davidson, D. A. and Spiers, R. B. (2000). Variability in contamination by heavy metals: Sampling implications. *Water Air and Soil Pollution* **120**: 29-45.

Angelone, M. and Bini, C. (1992). Trace element concentrations in soils and plants in western Europe. In: Biogeochemistry of trace metals. Adriano, D. C.(Eds.) Boca Raton, Lewis Publishers: 19-60.

Armstrong, A. (1997). The United Kingdom network of experiments on site/yield relationships for short rotation coppice. FC Research Information Note 294. Edinburgh, Forestry Commission.

Armstrong, A. (1999). Establishment and Management of Short Rotation Coppice. FCPN7. Edinburgh, Forestry Commission.

Armstrong, A., Johns, C. and Tubby, I. (1999). Effects of spacing and cutting cycle on the yield of poplar grown as an energy crop. *Biomass & Bioenergy* **17**: 305-314.

Arthur, E., Crews, H. and Morgan, C. (2000). Optimizing plant genetic strategies for minimizing environmental contamination in the food chain. *International Journal of Phytoremediation* **2**: 1-21.

Bacon, J. R. and Hudson, G. (2001). A flexible methodology for the characterisation of soils: a case study of the heavy metal status of a site at Dornach. *The Science of The Total Environment* **264**: 153-162.

Baker, A. J. M. (1981). Accumulators and excluders - strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**: 643.

Baker, A. J. M., McGrath, S. P., Reeeves, R. D. and Smith, J. A. C. (2000). Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. In: Phytoremediation of contaminated soil and water. Terry, N. and Banuelos, G.(Eds.) Boca Raton, FL., Lewis Publishers: 85-107.

Baker, D.E. and Senft, J. P. (1995). Copper. In: Heavy Metals in Soil. Alloway, B. J.(Eds.) Glasgow, Blackie.

Bardos, P., French, C. J., Moffat, A. J. and Nortcliff, S. (1999). Can biomass kill three birds with one stone? Renewable energy, contamination clean-up and organic waste recycling. *Land Contamination & Reclamation* **7**: 174-176.

Barton, M. R. (2000). Lessons learned, the Groundwork approach to delivering a large scale programme of brownfield land reclaimation. In: Conference Proceedings, Beyond Brownfields: Towards Equitable and Sustainable Development.(Eds.) New York, PICCED.

Baumann, A. (1885). Das Verhalten von Zinksatzen gegen Pflanzen und im Boden. *Landwirtsch. Vers.-Statn* **31**: 1-53.

Ben Brahim, M., Gavaland, A. and Cabanettes, A. (2000). Generalized allometric regression to estimate biomass of *Populus* in short - rotation coppice. *Scandinavian Journal of Forest Research* **15**: 171 - 176.

Bending, N. A. D., McRae, S. G. and Moffat, A. J. (1999). Soil-forming materials: their use in land restoration. London, The Stationary Office.

Bending, N. A. D. and Moffat, A. J. (1999). Tree performance on minespoils in the South Wales coalfield. *Journal of Applied Ecology* **36**: 784-797.

Berken, A., Mulholland, M. M., LeDuc, D. L. and Terry, N. (2002). Genetic Engineering of Plants to Enhance Selenium Phytoremediation. *Critical Reviews in Plant Sciences* **21**: 567-582.

Berndes, G., Fredrikson, F. and Borjesson, P. (2004). Cadmium accumulation and *Salix*-based phytoextraction on arable land in Sweden. *Agriculture, Ecosystems & Environment* **103**: 207-223.

Berry, C. R. (1985). Growth and heavy metal accumulation in Pine seedlings grown with sewage sludge. *Journal of Environmental Quality* **14**: 415 -419.

Berthelot, A., Ranger, J. and Gelhaye, D. (2000). Nutrient uptake and immobilization in a short rotation coppice stand of hybrid poplars in north-west France. *Forest Ecology and Management* **128**: 167 - 179.

Bhati, M. and Singh, G. (2003). Growth and mineral accumulation in *Eucalyptus camaldulensis* seedlings irrigated with mixed industrial effluents. *Bioresource Technology* **88**: 221-228.

Blaylock, M. J. and Huang, J. W. (1999). Phytoremediation of metals. In: Phytoremediation of toxic metals: Using plants to clean up the environment. Raskin, I. and Ensley, B. D.(Eds.) New York, USA, Wiley & Sons Inc.: 53-70.

Blaylock, M. J., Salt, D. E., Dushenkov, D. E., Zakharova, S., Gussman, O., Kapulnik, Y., Ensley, B. D. and Rasklin, I. (1997). Enhanced accumulation of Pb in Indian Mustard by soil applied chelating agents. *Environmental Science and Technology* **31**: 860-865.

Bondada, B. R., Ma, L. Q., Underhill, R. S., Duran, R. and Davidson, M. (2002). Spatial distribution of arsenic species in brake fern (*Pteris vittata* L.) using X-ray absorption spectroscopy. *Plant Biology Rockville* **2002**: 152.

Borovik, A. S. (1989). Charecterisation of metal ions in biological systems. In: Heavy Metal tolerance in plants: an evolutionary approach. Ed. Shaw, A. J., CRC Press.

Boudou, R. A. (1996). Linking plant tissue concentrations and soil copper pools in urban contaminated soils. *Environmental Pollution* **94**: 153-157.

Bradshaw, A. (1997). Restoration of mined lands using natural processes. *Ecological Engineering.* **8**: 255 - 269.

Bridgwater, A. V., Meier, D. and Radlein, D. (1999). An overview of fast pyrolysis of biomass. *Organic Geochemistry* **30**: 1479-1493.

Brooks, R. R. (1987). Serpentine and its vegetation. London, Croom Helm.

Brun, L. A., Maillet, J., Richarte, J., Herrmann, P. and Remy, J. C. (1998). Relationship betwen extractable copper, soil properties and copper uptake by wild plants in vineyard soils. *Environmental Pollution.* **102**: 151-161.

BSI (2001). Investigation of potentially contaminated sites - Code of practice. BS10175:2001, British Standards Institute.

BSI (2002). Soil Quality Sampling. Part 1. Guidence on the design of sampling programmes. BS ISO 10381-1:2002, British Standards Institute.

Bullard, M. J., Mustill, S. J., McMillan, S. D., Nixon, P. M. I., Carver, P. and P. Britt, C. (2002). Yield improvements through modification of planting density and harvest frequency in short rotation coppice *Salix* spp.--1. Yield response in two morphologically diverse varieties. *Biomass and Bioenergy* **22**: 15-25.

Burken, J. G. and Schnoor, J. L. (1997). Uptake and metabolism of atrazine by poplar trees. *Environmental Science and Technology* **31**: 1399.

Burken, J. G. and Schnoor, J. L. (1998). Predictive Relationships for uptake of Orgainic Contaminants by Hybrid Poplar trees. *Environ.Sci.Technol.* **32**: 3379-3385.

Cannell, M. G. R. (2003). Carbon sequestration and biomass energy offset: theoretical, potential and achievable capacities globally, in Europe and the UK. *Biomass and Bioenergy* **24**: 97-116.

Cataldo, D. A., Wildung, R. E. and Garland, T. R. (1987). Speciation of trace inorganic contaminants in plants and bioavailability to animals: an overview. *Journal of Environmental Quality* **16**: 289 - 295.

Chaney, R. L. (1983). Plant uptake of inorganic waste constituents. In: Land treatment of hazzardous wastes. Parr, J. F., Marsh, P. B. and Kla, J. M.(Eds.) Park Ridge, NJ, Noyes Data Corp.: 50-76.

Chaney, R. L., Li, Y.-M., Brown, S. L., Homer, F. A., Malik, M., Angle, J. S., Baker, A. J. M., Reeeves, R. D. and Chin, M. (2000). Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress. In: Phytoremediation of contaminated soil and water, CRC Press: 129-158.

Chen, B., Shan, X. Q. and Qian, J. (1996). Bioavailability index for quantitative evaluation of plant availability of extractable soil trace elements. *Plant and Soil* **186**: 275-283.

Chen, Z. S., Lee, G. J. and Liu, J. C. (2000). The effects of chemical remediation treatments on the extractability and speciation of cadmium and lead in contaminated soils. *Chemosphere* **41**: 235-242.

CL:AIRE (2004). Technical Bulletin TB7. Improving the reliability of contaminated land assessment using statistical methods: part 1 - basic principles and concepts. London, Contaminated Land: Applications in real environments.

Crow, P. and Houston, T. J. (2004). The influence of soil and coppice cycle on the rooting habit of short rotation poplar and willow coppice. *Biomass and Bioenergy* **26**: 497-505.

Davies, B. E. (1995). Lead. In: Heavy Metals in Soil. Alloway, B. J.(Eds.) Glasgow, Blackie.

Dawson, W. M. and McCracken, A. R. (1995). The Performance of Polyclonal Stands in Short-Rotation Coppice For Energy-Production. *Biomass & Bioenergy* **8**: 1-5.

DEFRA (2002a). Growing short rotation coppice - Best practice Guidelines. London.

DEFRA (2002b). Note on the withdrawal of ICRCL trigger values. Contaminated Land Advice Note (CLAN) 3/02, DEFRA.

DEFRA and Environment Agency (2002a). Assesment of risks to human health from land contamination: An overview of the development of soil guideline values and related research. CLR 7. Bristol, Environment Agency.

DEFRA and Environment Agency (2002b). Contaminants in soil: collation of toxicological data and intake values for humans. Lead. Bristol, Environment Agency.

DEFRA and Environment Agency (2002c). Contaminants in soil: collation of toxicological data and intake values for humans. Arsenic. Bristol, Environment Agency.

DEFRA and Environment Agency (2002d). Contaminants in soil: collation of toxicological data and intake values for humans. Cadmium. Bristol, Environment Agency.

DEFRA and Environment Agency (2002e). The contaminated land exposure assessment (CLEA) model: Technical basis and algorithms. CLR 10. Bristol, Environment Agency.

DEFRA and Environment Agency (2002f). Soil guideline values for cadmium contamination. SGV 3. Bristol, Environment Agency.

DEFRA and Environment Agency (2002g). Soil guideline values for Nickel contamination. Bristol, Environment Agency.

Devliegher, W. and Verstraete, W. (1996). *Lumbricus terrestris* in a soil core experiment: effects of nutrient enrichment processes (NEP) and gut-associated processes (GAP) on the availability of plant nutrients and heavy metals. *Soil Biology and Biochemistry* **28**: 489-496.

Devliegher, W. and Verstraete, W. (1997). The effect of *Lumbricus terrestris* on soil in relation to plant growth: Effects of nutrient-enrichment processes (NEP) and associated processes (GAP). *Soil Biology and Biochemistry* **29**: 341-346.

Dhankher, O. P., Li, Y., Rosen, B. P., Shi, J., Salt, D., Senecoff, J. F., Sashti, N. A. and Meagher, R. B. (2002). Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and gamma-glutamylcysteine synthetase expression. *Nature Biotechnology* **20**: 1140-1145.

Di Baccio, D., Tognetti, R., Sebastiani, L. and Vitagliano, C. (2003). Responses of *Populus deltoides x P. nigra* clone I-214 to high zinc concentrations. *New Phytologist* **159**: 443-452.

Dickinson, N. M. (2000). Strategies for sustainable woodland on contaminated soils. *Chemosphere* **41**: 259-263.

Dickinson, N. M., Mackay, J., Goodman, A. and Putwain, P. D. (2000). Planting trees on Contaminated Soils: Issues and Guidlines. *Land Contamination & Reclamation* **8**: 87-101.

Dobson, M. C. and Moffat, A. J. (1993). The potential for woodland establishment on landfill sites. London, HMSO.

Dobson, M. C. and Moffat, A. J. (1995). Research Information Note 263. Site capability assessment for woodland creation on landfills. Edinburgh, Forestry Commission.

DoE (1994). Contaminated Land Research Report CLR4: Sampling Strategies for Contaminated Land, Department of the Environment. (Prepared by The Centre for Research into the Built Environment, Nottingham Trent University).

DTI (2003). New and renewable energy - Prospects for the 21st Century. The Renewables Obligation (Amendment) Order 2003. Statutory Consultation. London, Department for Trade and Industry.

DTI (2004). Conclusions from the renewable innovations review. London, Department for Trade and Industry.

Dushenkov, S., Skarzhinskaya, M., Glimelius, K., Gleba, D. and Raskin, I. (2002). Bioengineering of a phytoremediation plant by means of somatic hybridization. *International Journal of Phytoremediation* **4**: 117-126.

Eltrop, L., Brown, G., Joachim, O. and Brinkmann, K. (1991). Lead tolerance of *Betula* and *Salix* in the mining area of Mechernich/Germany. *Plant and Soil* **131**: 275-285.

ENDS (2003). Co-firing plans in the spotlight over biomass imports. *ENDS Report* **342**: pp 24-27.

ENDS (2004). DTI innovation review seeks to plug gaps in renewables strategy. *ENDS Report* **350**: pp 31-33.

Environment Agency (2000). The State of the Environment of England and Wales: The Land. HMSO, London, Environment Agency.

Environment Agency (2002). Dealing with Contaminated Land in England:. Bristol. Environment Agency.

Environment Agency (2003). Ecological Risk Assessment: Public Consultation. Bristol, Environment Agency.

Eriksson, J. and Ledin, S. (1999). Changes in phytoavailability and concentration of cadmium in soil following long term *Salix* cropping. *Water Air and Soil Pollution* **114**: 171-184.

Ernst, W. H. O. (1996). Bioavailability of heavy metals and decontamination of soils by plants. *Applied Goechemistry* **11**: 163-167.

exSite Research (2001). Marginal land Restoration Scoping Study: Information review and Feasibility Study. Nottingham, UK, Land Quality Press.

Felix, H. (1997). Field trials for in situ decontamination of heavy metal potlluted soils using crops of metal-accumulating plants. *Z. Pflanzenernahr Bodenk* **160**: 525-529.

Fitz, W. J., Wenzel, W. W., Zhang, H., Nurmi, J., Stipek, K., Fischerova, Z., Schweiger, P., Kollensperger, G., Ma, L. Q. and Stingeder et, a. (2003). Rhizosphere characteristics of the arsenic hyperaccumulator *Pteris vittata* L. and monitoring of phytoremoval efficiency. *Environmental Science & Technology* **37**: 5008-5014.

Forest Research (2003). Preliminary Yield Estimates, HMSO.

Forestry Commission (1991). Forestry Practice. London, UK, HMSO.

Forestry Commission (2003). Roots - The software for sustainable growth. LRU News. **2**.

Francesconi, K., Visoottiviseth, P., Sridokchan, W. and Goessler, W. (2002). Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of arsenic-contaminated soils. *The Science of the Total Environment.*: 27-35.

Freer-Smith, P. H., Holloway, S. and Goodman, A. (1997.). The uptake of particulates by an urban woodland: site description and particulate composition. *Environmental Pollution.* **95**: 27 - 35.

Friend, A. L., G. Scarascia-Mugnozza, Isebrands, J. G. and Heilman., P. E. (1991). Quantification of two-year-old hybrid poplar root systems: morphology, biomass, and C14 distribution. *Tree Physiology* **8**: 109-119.

Garbisu, C. and Alkorta, I. (2001). Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. *Bioresource Technology* **77**: 229-236.

Gerhardt Ltd (2002). Kjeldahl Distillation: Application Note PAN 10.

Gisbert, C., Ros, R., De Haro, A., Walker, D. J., Pilar Bernal, M., Serrano, R. and Navarro-Avino, J. (2003). A plant genetically modified that accumulates Pb is especially promising for phytoremediation. *Biochemical and Biophysical Research Communications* **303**: 440-445.

Gommers, A., Thiry, Y., Vandenhove, H. and Vandecasteele, C. M. (2000). Radiocesium Uptake by One-Year-Old Willows Planted as Short Rotation Coppice. *Journal of Environmental Quality* **29**: 1384-1390.

Goor, F., Davydchuk, V. and Ledent, J.-F. (2001). Assessment of the potential of willow SRC plants for energy production in areas contaminated by radionuclide deposits: methodology and perspectives. *Biomass and Bioenergy* **21**: 225-235.

Goransson, A. and Philippot, S. (1994). The use of fast growing trees as 'metal-collectors'. In: Willow vegetation filters for municipal watstewaters and sludges: a biological purification system. Aronsson, P. and Perttu, K.(Eds.) Uppsala, Sweden, Sveriges Lantbruksuniversitet. **Report 50:** 129-132.

Greger, M. and Landberg, T. (1997). Use of Willow clones with high Cd accumulating properties in phytoremediation of agricultural soils with eleveated Cd levels in Contaminated Soils. In: Procedings of ICOBTE 3 Paris 1995. Prost, R.(Eds.) Paris.

Greger, M. and Landberg, T. (1999). Use of willows in phytoextraction. *International Journal of Phytoremediation* **1**: 115-123.

Grimski, D. and Ferber, U. (2001). Urban Brownfield in Europe. *Land contamination and reclamation* **9**: 143-148.

Haines, B. J. (2002). Zincophilic root foraging in *Thlaspi caerulescens*. *New Phytologist* **155**: 363-372.

Hasselgren, K. (1998). Use of municipal waste products in energy forestry: highlights from 15 years of experience. *Biomass and Bioenergy* **15**: 71-74.

Heinsoo, K., Sild, E. and Koppel, A. (2002). Estimation of shoot biomass productivity in Estonian *Salix* plantations. *Forest Ecology and Management* **170**: 67-74.

Herve, C. and Ceulemans, R. (1996). Short-rotation coppiced vs non-coppiced poplar: A comparative study at two different field sites. *Biomass and Bioenergy* **11**: 139-150.

Hetland, M. D., Gallagher, J. R., Daly, D. J., Hassett, D. J. and Heebink, L. V. (2001). Processing of plants used to phytoremediate lead-contaminated sites. In: Phytoremediation, Wetlands, and Sediments, The 6th International in situ and on site bioremediation symposium. Leeson, A., Foote, E. A., Banks, M. K. and Magar, V. S.(Eds.) San Diego, California, Battelle Press, Columbus, Richland: 129-136.

Houba, V. J. G., Lexmond, T. M., Novozamsky, I. and van der Lee, J. J. (1996). State of the art and future developments in soil analysis for bioavailability assessment. *The Science of the Total Environment.* **178**: 21-28.

Huang, J. W., Chen, J. J., Berti, W. R. and Cunningham, S. D. (1997). Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoexraction. *Environmental Science and Technology* **31**: 800-805.

Hutchings, T. R. (2002). The opportunities for woodland on contaminated land: Information Note 44. Edinburgh, UK, Forestry Commission.

Hutchings, T. R., Moffat, A. J. and Kemp, R. A. (2001). Effects of rooting and tree growth of selected woodland species on cap integrity in a mineral capped landfill site. *Waste Management & Research: The Journal Of The International Solid Wastes And Public Cleansing Association, ISWA* **19**: 194-200.

ICRCL (1987). Inter-Departmental Committee on the Redevelopment of Contaminated Land: Guidance Notes (59/83). London, HMSO.

IEMA (2004). End of co-disposal of hazardous and non hazardous waste. *The Environmentalist* **24**: 10-11.

Ireland, M. P. (1975). The effect of the eartthworm *Dendrobaena rubida* on the solubility of lead, zinc and calcium in heavy metal contaminated soil in Wales. *Journal of Soil Science* **26**: 313-318.

Jarvis, M. D. and Leung, D. W. M. (2002). Chelated lead transport in *Pinus radiata*: an ultrastructural study. *Environmental and Experimental Botany* **48**: 21-32.

Johansson, T. (2000). Biomass equations for determining fractions of common and grey alders growing on abandoned farmland and some practical implications. *Biomass & Bioenergy* **18**: 147-159.

Kabata-Pendias, A. (2001). Trace Elements in Soils and Plants, 3rd edn. Boca Raton, Florida, CRC Press.

Kahle, H. (1993). Response of roots of trees to heavy metals. *Environmental and Experimental Botany* **33**: 99-119.

Kavanagh, P. J. (1997). Bioavailability of arsenic in soil and mine wastes of the Tamar Valley, SW England. *Chemical Speciation and Bioavailability* **9**: 77-81.

Khan, A. G., Kuek, C., Chaudhry, T. M., Khoo, C. S. and Hayes, W. J. U. (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere* **41**: 197-207.

Kieffer, F. (1991). Metals and their compounds in the Environment. Merian, E.(Ed.) Weinheim, VCH.

Kiekens, L. (1995). Zinc. In: Heavy Metals in Soil. Alloway, B. J.(Eds.) Glasgow, Blackie.

Klang-Westin, E. and Eriksson, J. (2003). Potential of *Salix* as phytoextractor for Cd on moderately contaminated soils. *Plant and Soil* **249**: 127-137.

Klein, M. A., Pence, N. S. and Kochian, L. V. (2002). Characterization of zinc tolerance genes in the zinc/cadmium hyperaccumulator *Thlaspi caerulescens*. *Plant Biology Rockville* **2002**: 154.

Koppolu, L., Agblevor, F. A. and Clements, L. D. (2003). Pyrolysis as a technique for separating heavy metals from hyperaccumulators. Part II: Lab-scale pyrolysis of synthetic hyperaccumulator biomass. *Biomass and Bioenergy* **25**: 651-663.

Kozlov, M. V., Haukioja, E., Bakhtiarov, A. V., Stroganov, D. N. and Zimina, S. N. (2000). Root versus canopy uptake of heavy metals by birch in an industrially polluted area: contrasting behaviour of nickel and copper. *Environmental Pollution* **107**: 413-420.

Kumar, P. B. A. N., Dushenkov, V., Motto, H. and Raskin, I. (1995). Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental Science and Technology* **29**: 1232-1238.

Lai, H. Y. and Chen, Z. S. (2004). Effects of EDTA on solubility of cadmium, zinc, and lead and their uptake by rainbow pink and vetiver grass. *Chemosphere* **55**: 421-430.

Landberg, T. and Greger, M. (1994). Heavy metal tolerant clones of *Salix* : possible vegetation filter on heavy metal contaminated land. In: Willow vegetation filters for municipal wastewaters and sludges: a biological purification system. Perttu, K. L. and Aronsson, P.(Eds.) Sweden.

Langdon, C. J., Piearce, T. G., Black, S. and Semple, K. T. (1999). Resistance to arsenic-toxicity in a population of the earthworm *Lumbricus rubellus*. *Soil Biology and Boichemistry*. **31**: 1963-1967.

Langdon, C. J., Piearce, T. G., Meharg, A. A. and Semple, K. T. (2001). Survival and behaviour of the earthworms *Lumbricus rubellus* and *Dendrodrilus rubidus* from arsenate-contaminated and non-contaminated sites. *Soil Biology and Biochemistry*. **33**: 1239-1244.

Laureysens, I., Bogaert, J., Blust, R. and Ceulemans, R. (2004). Biomass production of 17 poplar clones in a short-rotation coppice culture on a waste disposal site and its relation to soil characteristics. *Forest Ecology and Management* **187**: 295-309.

Laureysens, I., Deraedt, W., Indeherberge, T. and Ceulemans, R. (2003). Population dynamics in a 6-year old coppice culture of poplar. I. Clonal differences in stool mortality, shoot dynamics and shoot diameter distribution in relation to biomass production. *Biomass and Bioenergy* **24**: 81-95.

le Clercq, M., Adschiri, T. and Arai, K. (2001). Hydrothermal processing of nickel containing biomining or bioremediation biomass. *Biomass and Bioenergy* **21**: 73-80.

Le Duc, M. G., Pakeman, R. J. and Marrs, R. H. (in press). Extracting information from noisy survey data on temporal change in vegetation following disturbance. *Ecological Modelling.*

LEK Consulting (2004). A review of the economic case for energy crops in the UK (for DTI). London.

Lim, J.-M., Salido, A. L. and J. Butcher, D. (2004). Phytoremediation of lead using Indian mustard (*Brassica juncea*) with EDTA and electrodics. *Microchemical Journal* **76**: 3-9.

Ma, L. Q., Komar, K. M., Tu, C., Zhang, W. and Cai, Y. (2001). A fern that hyperaccumulates Arsenic. *Nature* **409**: 579.

Ma, Y., Dickinson, N. M. and Wong, M. H. (2003). Interactions between earthworms, trees, soil nutrition and metal mobility in amended Pb/Zn mine tailings from Guangdong, China. *Soil Biology and Biochemistry* **2003**: 1369-1379.

Macnair, M. R. (1993). Tansley Review No 49 : The genetics of metal tolerance in vascular plants. *New Phytologist* **124**: 541 - 559.

MAFF (1993). Review of the rules for sewage sludge application to agricultural land: soil fertility aspects of potentially toxic elements. London, MAFF Publications.

Maiz, I., Esnaola, M. V. and Millan, E. (1997). Evaluation of heavy metal availability in contaminated soils by a short sequential extraction procedure. *The Science of the Total Environment.* **206**: 107-115.

Makeschin, F. (1999). Short Rotation Forestry in centeral and Northern Europe - Introduction and Conclusions. *Forest Ecology and Management* **121**: 1-7.

Martino, E., Perotto, S., Parsons, R. and Gadd, G. M. (2003). Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. *Soil Biology and Biochemistry* **35**: 133-141.

Mattusch, J., Wennrich, R., Schmidt, A. C. and Reisser, W. (2000). Determination of arsenic species in water, soils and plants. *Fresenius Journal of Analytical Chemistry* **366**: 200-203.

Maxted, A. P. (2002). Phytoextraction of cadmium from soils treated with sewage sludge. PhD Thesis, University of Nottingham.

McCracken, A. R., Dawson, W. M. and Bowden, G. (2001). Yield responses of willow (*Salix*) grown in mixtures in short rotation coppice (SRC). *Biomass and Bioenergy* **21**: 311-319.

McGrath, S. P. (1994). Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States. *Environmental Reviews* **2**.

McGrath, S. P. (1995). Chromium and Nickel. In: Heavy Metals in Soil. Alloway, B. J.(Eds.) Glasgow, Blackie.

McGrath, S. P. and Loveland, P. J. (1992). The soil geochemical atlas of England and Wales. London, Blackie Academic and Professional.

Meharg, A. A. and Cairney, W. G. (2000). Ectomycorrhizas - extending the capabilities of rhizosphere remediation. *Soil Biology and Biochemistry* **32**: 1475 - 1484.

Mehra, A. and Farago, M. E. (1994). Metal Ions and Plant Nutrition. In: Plants and the Chemical Elements. Farago, M. E.(Eds.) New York, VCH.

Mersey Forest (1999a). The assessment of brownfield site for community woodland use: Merton Bank. Report No RO2d. Warrington, Prepared by Allot and Lomax Consulting Engineers.

Mersey Forest (1999b). Creating Community Woodlands on Closed Landfill Sites. Warrington, The Mersey Forest.

Milligan, A. L., Putwain, P. D., Cox, E. S., Ghorbani, J., Le Duc, M. G. and Marrs, R. H. (2004). Developing an integrated land management strategy for the restoration of moorland vegetation on *Molinia caerulea* dominated vegetation for conservation purposes in upland Britain. *Biological Conservation* **119**: 371-385.

Mitchell, C. P., Stevens, E. A. and Watters, M. P. (1999). Short rotation forestery-operations productivity and costs based on the experiance in the UK. *Forest Ecology and Management* **121**: 123-136.

Mitchell, P. and Barr, D. (1995). The nature and significance of public exposure to Arsenic: A review of its relevence to SW England. *Environmental Geochemistry and Health* **17**: 57-82.

Moffat, A. J. (2000). Effects of inoculation with *Frankia* on the growth and nutrition of alder species and interplanted Japanese larch on restored mineral workings. *Forestry* **73**: 215-223.

Moffat, A. J., Armstrong, A. T. and Ockleston, J. (2001.). The optimization of sewage sludge and effluent disposal on energy crops of short rotation hybrid poplar. *Biomass and Bioenergy.* **20**: 161 -169.

Moffat, A. J. and McNeil, J. D. (1994). Reclaiming disturbed land for forestry. Forestry Commission Bulletin 110. London, HMSO.

Moffat, A. J. and McNeill, J. D. (1992). Alders - valuable componenents of woodland on reclaimed land. *Mineral Planning* **53**: 23-24.

Moffatt, A. J. (1995). Minimum soil depths for the establishment of woodland on disturbed ground. *Arboricultural Journal* **19**: 19-27.

Mulligan, C. N., Yong, R. N. and Gibbs, B. F. (2001). Remediation technologies for metal-contaminated soils and groundwater: an evaluation. *Engineering Geology* **60**: 193-207.

Murphy, D. T. (1979). The reclamation of alkali waste: Merton Bank, a case study. Department of Geography. Liverpool, The University of Liverpool.

Newman, L. A., Strand, S. S., Choe, N., Duffy, J., Ekuan, G., Ruszaj, M., Shurtleff, B. B., Wilmoth, J., Heilman, P. and Gordon, M. P. (1997). Uptake and biotransformation of trichloroethylene by hybrid poplars. *Environmental Science and Technology* **31**: 1062-1067.

Nissen, L. R. and Lepp, N. W. (1997). Baseline concentrations of copper and zinc in shoot tissues of a range of *Salix* species. *Biomass and Bioenergy* **12**: 115-120.

Nissen, L. R., Lepp, N. W. and Edwards, R. (2000). Synthetic zeolites as amendments for sewage sludge-based compost. *Chemosphere* **41**: 265-269.

Nixon, D. J., Stephens, W., Tyrrel, S. F. and Brierley, E. D. R. (2001). The potential for short rotation energy forestry on restored landfill caps. *Bioresource Technology* **77**: 237-245.

Nolan, A. L., Lombi, E. and McLaughlin, M. J. (2003). Metal bioaccumulation and toxicity in soils - why bother with speciation?'. *Australian Journal of Chemistry* **56**: 77-91.

Nortcliff, S. (2001). Sampling and pre-treatment -- some observations from the United Kingdom. *The Science of The Total Environment* **264**: 163-168.

Novozamsky, I., Lexmond, T. M. and Houba, V. J. G. (1993). A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. *International Journal of Environmental and Analytical Chemistry* **51**: 47-58.

Nriagu, J. O. (1989). A global assesment of natural sources of atmospheric trace metals. *Nature* **338**: 47-49.

NWDA (2003). Newlands - New economic environments through woodlands, North West Development Agency.

O'Neill, P. (1995). Arsenic. In: Heavy Metals in Soil. Alloway, B. J.(Eds.) Glasgow, Blackie.

Orlandi, M., Pelfini, M., Pavan, M., Santilli, M. and Colombini, M. P. (2002). Heavy metals variations in some conifers in Valle d'Aosta (Western Italian Alps) from 1930 to 2000. *Microchemical Journal* **73**: 237-244.

Ostman, G. (1994). Cadmium in *Salix* - a study of the capacity of *Salix* to remove cadmium from arable soils. In: Willow Vegetation Filters for Municipal Waste Waters and Sludges: A Biological Purification System. Aronnsson, P. and Perttu, K.(Eds.) Uppsala, Swedish University of Agricultural Sciences: 153-155.

Pahlsson, A.-M. B. (1989). Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants: a literature review. *Water, Air and Soil Polution* **47**: 287-319.

Panwar, B. S., Ahmed, K. S. and Mittal, S. B. (2002). Phytoremediation of nickel-contaminated soils by *Brassica* species. *Environment Development and Sustainability* **4**: 1-6.

Paulson, M., Bardos, P., Harmsen, J., Wilczek, J., Barton, M. and Edwards, D. (2003). The practical use of short rotation coppice in land restoration. *Land contamination and reclaimation* **11**: 232 - 338.

Pellis, A., Laureysens, I. and Ceulemans, R. (2004). Growth and production of a short rotation coppice culture of poplar I. Clonal differences in leaf characteristics in relation to biomass production. *Biomass and Bioenergy* **27**: 9-19.

Perry, D. and Handley, J. (2000). Forestry Commission Technical Paper 29: The potential for woodland on urban and industrial wasteland in England and Wales. Edinburgh, Forestry Commission.

Perttu, K. (1993). Biomass production and nutrient removal from municipal wastes using willow vegetation filters. *Journal of Sustainable Forestry* **1**: 57-70.

Perttu, K. L. (1998). Environmental justification for short-rotation forestry in Sweden. *Biomass & Bioenergy* **15**: 1-6.

Perttu, K. L. (2002). Content and fluxes of cadmium in the soil-*Salix* system (In Swedish, with English summary). Uppsala, Statens energimyndighet.

Pilon-Smits, E. and Pilon, M. (2000). Breeding mercury-breathing plants for environmental cleanup. *Trends in Plant Science* **5**: 235-236.

Pilon-Smits, E. and Pilon, M. (2002). Phytoremediation of metals using transgenic plants. *Critical Reviews in Plant Sciences* **21**: 439-456.

Pollard, A. J., Powell, K. D., Harper, F. A. and Smith, J. A. C. (2002). The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences* **21**: 539-566.

Pollard, J. A. and Baker, A. J. M. (1997). Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens*. *New Phytol.* **135**: 655 - 658.

Pongratz, R. (1998). Arsenic speciation in environmental samples of contaminated soil. *The Science of The Total Environment* **224**: 133-141.

Pueyo, M., Lopez-Sanchez, J. F. and Rauret, G. (2003). Assessment of $CaCl_2$, $NaNO_3$ and NH_4NO_3 extraction procedures for the study of Cd, Cu, Pb and Zn extractability in contaminated soils. *Analytica Chimica Acta* **504**: 217-226.

Pulford, I. D., Riddell, B. D. and Stewart, C. (2002). Heavy metal uptake by willow clones from sewage sludge-treated soil: The potential for phytoremediation. *International Journal of Phytoremediation* **4**: 59-72.

Pulford, I. D. and Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees--a review. *Environment International* **29**: 529-540.

Punshon, T. and Dickinson, N. M. (1997). Mobilisation of heavy metals using short - rotation coppice. *Aspects of Applied Biology* **49**: 285-292.

Punshon, T. and Dickinson, N. M. (1999). Heavy metal resistance and accumulation characteristics in willows. *International Journal of Phytoremediation* **1**: 361-385.

Putwain, P. D., Rawlinson, H. A., French, C. J., Dickinson, N. M. and Nolan, P. (2003). The Mersey Forest Brownfield Research Project. In: Land Reclamation: Extending the Boundaries. Moore, H. M., Fox, H. R. and Elliott, S.(Eds.) Lisse. The Netherlands, A.A. Balkema: 165-172.

Pyatt, F. B. (2001). Copper and lead bioaccumulation by *Acacia retinoides* and *Eucalyptus torquata* in sites contaminated as a consequence of extensive ancient mining activities in cyprus. *Ecotoxicology and Environmental Safety* **50**: 60-64.

Quevauviller, P., Lachica, M., Barahona, E., Rauret, G., Ure, A., Gomez, A. and Muntau, H. (1996a). Interlaboratory comparison of EDTA and DTPA procedures prior to certification of extractable trace elements in calcareous soil. *The Science of The Total Environment* **178**: 127-132.

Quevauviller, P., van der Sloot, H. A., Ure, A., Muntau, H., Gomez, A. and Rauret, G. (1996b). Conclusions of the workshop: harmonization of leaching/extraction tests for environmental risk assessment. *The Science of The Total Environment* **178**: 133-139.

Ramsey, M. H., Taylor, P. D. and Lee, J.-C. (2002). Optimized contaminated land investigation at minimum overall cost to achieve fitness-for-purpose. *Journal Of Environmental Monitoring: JEM* **4**: 809-814.

Rate, A. W., Lee, K. M. and French, P. A. (2004). Application of biosolids in mineral sands mine rehabilitation: use of stockpiled topsoil decreases trace element uptake by plants. *Bioresource Technology* **91**: 223-231.

Rawlinson, H., Putwain, P. D. and Dickinson, N. M. (2000). Constraints to the establishment of community woodlands on closed landfill sites. *Aspects of Applied Biology* **58**: 123-128.

Rawlinson, H. A. (2001). The establishment of community woodlands on closed landfill sites. Ph.D. Thesis. School of Biological Sciences. Liverpool, University of Liverpool.

Reeves, R. D. (1992). Hyperaccumulation of Ni by serpintine plants. In: The vegetation of ultramafic (serpentine) soils. Baker, A. J. M., Proctor, J. and Reeves, R. D.(Eds.) Andover, Hampshire, Intercept Ltd.: 253-277.

Reeves, R. D. and Brooks, R. R. (1983). Hyperaccumulation of lead and zinc by two metallophytes from mining areas of central Eurpoe. *Environmental Pollution* **31**: 277-285.

Rentz, J. A., Chapman, B., Alvarez, P. J. J. and Schnoor, J. L. (2003). Stimulation of hybrid poplar growth in petroleum-contaminated soils through oxygen addition and soil nutrient amendments. *International Journal of Phytoremediation* **5**: 57-72.

Ricklefs, R. E. and Nealan, P. (1998). Lineage-dependant rates of evolutionary diversification: analysis of bivatiate ellipses. *Functional Ecology* **12**: 871-888.

Riddell-Black, D. (1994). Heavy Metal Uptake by Fast Growing willow Species. In: Willow vegetation filters for municipal wastewaters and sludges. A Biological Purification system. Aronsson, P. and Perttu, K. L.(Eds.) Upsala, Sweden, Swedish University of Agricultural Sciences: 145-151.

Riddell-Black, D. (1995). Fertilisation of Short Rotation Energy Coppice using Sewage Sludge, Wrc plc.

Riddell-Black, D. (2002). BIORENEW. Bioremediation and Economic renewal of Industrially Degraded Land by Biomass Fuel Crops: Summary Report. Swindon, WRc Swindon.

Riddell-Black, D., Pulford, I. D. and Stewart, C. (1997). Clonal variation in heavy metal uptake by willow. *Aspects of Applied Biology* **49**: 327-334.

Robinson, B. H., Brookes, R. R. and Clothier, B. E. (1999). Soil amendments affecting nickel and cobalt uptake by *Berkheya coddii* : potential use for phytomining and phytoremediation. *Annals of Botany* **84**: 689-694.

Robinson, B. H., Brooks, R. R., Howes, A. W., Kirkman, J. H. and Gregg, P. E. H. (1997). The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining. *Journal of Geochemical Exploration* **60**: 115-126.

Robinson, B. H., Mills, T. M., Petit, D., Fung, L. E., Green, S. R. and Clothier, B. E. (2000). Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. *Plant and Soil* **227**: 301-306.

Rosenqvist, H., Roos, A., Ling, E. and Hektor, B. (2000). Willow growers in Sweden. *Biomass & Bioenergy* **18**: 137-145.

Rosselli, W., Keller, C. and Boschi, K. (2003). Phytoextraction capacity of trees growing on a metal-contaminated soil. *Plant and Soil* **256**: 265 - 272.

Rugh, C. L. (2000). Phytoreduction of environmental mercury pollution. In: Phytoremediation of toxic metals: Using plants to clean up the environment. Raskin, I. and Ensley, B. D.(Eds.) New York, USA, Wiley & Sons Inc.: 151-171.

Ruiz, O. N., Hussein, H. S., Terry, N. and Daniell, H. (2003). Phytoremediation of Organomercurial Compounds via Chloroplast Genetic Engineering. *Plant Physiology* **132**: 1344-1352.

Rytter, R. M. (1999). Fine-root production and turnover in a willow plantation estimated by different calculation methods. . *Scandinavian Journal of Forest Research* **14**: 526-537.

Rytter, R.-M. (1997). Fine root production and carbon and nitrogen allocation in basket willows. Department of Short Rotation Forestry. Uppsala, Swedish University of Agricultural Sciences.

Salido, A. L., Hasty, K. L., Lim, J.-M. and Butcher, D. J. (2003). Phytoremediation of arsenic and lead in contaminated soil using Chinese brake ferns (*Pteris vittata*) and Indian mustard (*Brassica juncea*). *International Journal of Phytoremediation* **5**: 89-103.

Sander, M. L. and Ericsson, T. (1998). Vertical distributions of plant nutrients and heavy metals in *Salix viminalis* stems and their implications for sampling. *Biomass & Bioenergy* **14**: 57-66.

Sarret, G., Vangronsveld, J., Manceau, A., Musso, M., D'Haen, J., Menthonnex, J.-J. and Hazemann, J.-L. (2001). Accumulation Forms of Zn and Pb in *Phaseolus vulgaris* in the Presence and Absence of EDTA. *Environmental Science & Technology* **35**: 2854-2859.

Sas-Nowosielska, A., Kucharski, R., Malkowski, E., Pogrzeba, M., Kuperberg, J. M. and Krynski, K. (2004). Phytoextraction crop disposal--an unsolved problem. *Environmental Pollution* **128**: 373-379.

Schmidt, U. (2003). Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. *Journal of Environmental Quality* **32**: 1939-1954.

Schnoor, J. L. (1997). Phytoremediation - Technology Evaluation Report. Pittsburgh, USA, Groundwater Remediation Technologies Analysis Centre.

Sebastiani, L., Scebba, F. and Tognetti, R. (2004). Heavy metal accumulation and growth responses in poplar clones Eridano (*Populus deltoides x maximowiczii*) and I-214 (*P. x euramericana*) exposed to industrial waste. *Environmental and Experimental Botany* **52**: 79-88.

Shang, T. Q. and Gordon, M. P. (2002). Transformation of [$_{14}$C] trichloroethylene by poplar suspension cells. *Chemosphere* **47**: 957-962.

Simms, D. L. and Beckett, M. J. (1987). Contaminated land: Setting trigger concentrations. *The Science of The Total Environment* **65**: 121-134.

Sommerville, A. H. C. (1992). Willows in the environment. In: Proceedings of the conference, Willow symbiosis. Watling, R. and Ravens, J. A.(Eds.) Royal Botanic Gardens, Edinburgh. **98B:** 215-224.

Song, J., Zhao, F.-J., Luo, Y.-M., McGrath, S. P. and Zhang, H. (2004). Copper uptake by *Elsholtzia splendens* and *Silene vulgaris* and assessment of copper phytoavailability in contaminated soils. *Environmental Pollution* **128**: 307-315.

Steer, P. (1997). Colliery spoil, sewage and biomass - potential for renewable energy from wastes. *Aspects of Applied Biology* **49**: 300 - 305.

Tabbush, P. and Beaton, A. (1998). Hybrid poplars: present status and potential in Britain. *Forestry.* **71**: 355-364.

Tabbush, P. and Lonsdale, D. (1999). Approved Poplar Varieties: Information Note 21. Edinburgh, UK, Forestry Commission.

Tabbush, P. and Parfitt, R. (1999). Poplar and willow varieties for short rotation coppice. Edinburgh, Forestry Commission.

The Countryside Agency (2003). Community Forest Programme. Evaluation overview - a report by the Countryside Agency. London, The Countryside Agency.

Tubby, I. and Armstrong, A. (2002). Establishment and Management of Short Rotation Coppice. FCPN7 (Revised). Edinburgh, Forestry Commission.

Turner, A. P. and Dickinson, N. M. (1993). Survival of *Acer pseudoplatanus* L. (sycamore) seedlings on metalliferous soils. *New Phytologist* **123**: 509-521.

UN/ECE-EC. (1998). Manual on methods and criteria for hamonised sampling, assessment, monitoring and analysis of the effects of air pollution on forests.

UNEP / UNECE (1997a). United Nations Economic Commission for Europe. Methods and criteria for harmonized sampling and assesment. Part III. Sampling and Analysis of Soil. UNEP Method 9101SA (PSD), UNEP / UNECE.

UNEP / UNECE (1997b). United Nations Economic Commission for Europe. Methods and criteria for harmonized sampling and assesment. Part III. Sampling and Analysis of Soil. UNEP Method 9103SA (pH), UNEP / UNECE.

Ure, A. M. (1996). Single extraction schemes for soil analysis and related applications. *The Science of The Total Environment* **178**: 3-10.

Uri, V., Tullus, H. and Lohmus, K. (2002). Biomass production and nutrient accumulation in short-rotation grey alder (*Alnus incana* (L.) Moench) plantation on abandoned agricultural land. *Forest Ecology and Management* **161**: 169-179.

USEPA (1994). Microwave assisted acid digestion of sediments, sludges, oils and soils. Method 3051, United States Environmental Protection Agency.

USEPA (1998). A Citizens Guide to Phytoremediation. EPA 542-F-98-011, United States Environmental Protection Agency.

Utriainen, M. A., Karenlampi, L. V., Karenlampi, S. O. and Schat, H. (1997). Differential tolerance to copper and zinc of micropropagated birches tested in hydroponics. *New Phytologist* **137**: 543-549.

Van Assche, F. and Clijsters, H. (1990). Effects of metals on enzyme activity in plants. *Plant, Cell and Environment* **13**: 195-206.

van Straalen, N. M. (2002). Assessment of soil contamination--a functional perspective. *Biodegradation* **13**: 41-52.

Van Tichelen, K. K., Colpaert, J. V. and Vangronsveld, J. (2001). Ectomycorrhizal protection of *Pinus sylvestris* against copper toxicity. *New Phytologist* **150**: 203-213.

Vandecasteele, B., De Vos, B. and Tack, F. M. G. (2002). Cadmium and Zinc uptake by volunteer willow species and elder rooting in polluted dredged sediment disposal sites. *The Science of The Total Environment* **299**: 191-205.

Vervaeke, P., Luyssaert, S., Mertens, J., De Vos, B., Speleers, L. and Lust, N. (2001). Dredged sediment as a substrate for biomass production of willow trees established using the SALIMAT technique. *Biomass & Bioenergy* **21**: 81-90.

Vervaeke, P., Luyssaert, S., Mertens, J., Meers, E., Tack, F. M. G. and Lust, N. (2003). Phytoremediation prospects of willow stands on contaminated sediment: a field trial. *Environmental Pollution* **126**: 275-282.

Verwijst, T. and Telenius, B. (1999). Biomass estimation procedures in short rotation forestry. *Forest Ecology and Management* **121**: 137-146.

Volk, T. A., L.P. Abrahamson and White, E. H. (2001). Root Dynamics in Willow Biomass Crops. Interim Report. Prepared for the United States Department of Energy Under Cooperative Agreement No. DE-FC36-96GO10132, State University of New York. College of Environmental Science & Forestry.

Ward, N. (1996). The place of trees in the reclamation of disturbed land. In: Forestry Commission Technical Paper 22: Recycling land for forestry. Proceedings of the Forestry Commission and BLRS conference. Moffat, A. J.(Eds.) University of Wolverhampton, Forestry Commission. Edinburgh: 1-3.

Wasay, S. A., Parker, W., Geel, P. J. V. and Tokunaga, S. (2000). Arsenic Pollution of a Loam Soil: Retention Form and Decontamination. *Journal of Soil Contamination.* **9**: 51-64.

Watmough, S. A. and Dickinson, N. M. (1995). Dispersal and mobility of heavy metals in relation to tree survival in an aerially contaminated woodland soil. *Environmental Pollution.* **90**: 135 - 142.

Watmough, S. A. and Hutchinson, T. C. (2004). The quantification and distribution of pollution Pb at a woodland in rural south central Ontario, Canada. *Environmental Pollution* **128**: 419-428.

Watson, C., Pulford, I. D. and Riddell-Black, D. (2003). Screening of willow species for resistance to heavy metals: comparison of performance in a hydroponics system and field trials. *International Journal of Phytoremediation* **5**: 351-365.

Wilkins, C. (1997). The uptake of copper arsenic and zinc by *Miscanthus*: environmental implications for use as an energy crop. *Aspects of Applied Biology* **49**: 335-340.

Wilkinson, D. M. and Dickinson, N. M. (1994). Metal resistance in trees: the role of mycorrhizae. *Oikos* **72**: 298-300.

Willoughby, I. and Clay, D. (1999). Herbicide Update. Edinburgh, Forestry Commission.

Wolfe, A. K. and Bjornstad, D. J. (2002). Why Would Anyone Object? An Exploration of Social Aspects of Phytoremediation Acceptability. *Critical Reviews in Plant Sciences* **21**: 429-438.

Wolstenholme, R., Dutch, J., Moffat, A. J., Bayes, C. D. and Taylor, C. M. A. (1992). A manual of good practice for the use of sewage sludge in forestry, Forestry Commission Bulletin, Vol 107. London, HMSO.

WS Atkins and Environment Agency (1998). Proposed wetland area at Fazakerley, Liverpool. Consultation Document ref: AY2366.120/JMH/kat/120.14236. Warrington.

Wu, L. H., Luo, Y. M., Xing, X. R. and Christie, P. (2004). EDTA-enhanced phytoremediation of heavy metal contaminated soil with Indian mustard and associated potential leaching risk. *Agriculture, Ecosystems & Environment* **102**: 307-318.

Yoon, J. M., Oh, B. T., Just, C. L. and Schnoor, J. L. (2002). Uptake and leaching of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine by hybrid poplar trees. *Environmental Science and Technology* **36**: 4649-4655.

Zhang, W., Cai, Y., Tu, C. and Ma, L. Q. (2002). Arsenic speciation and distribution in an arsenic hyperaccumulating plant. *The Science of The Total Environment* **300**: 167-177.

Zhao, F. J., Hamon, R. E. and McLaughlin, M. J. (2001). Root exudates of the hyperaccumulator *Thlaspi caerulescens* do not enhance metal mobilization. *New Phytologist* **151**: 613-620.

Zhao, F. J., Lombi, E., Breedon, T. and McGrath, S. P. (2000). Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. *Plant, Cell and Environment* **23**: 507-514.

Appendix 1 – CD-ROM containing MS Excel spreadsheets showing all plot planting designs