Phytoremediation of metal-contaminated soils by industrial crops

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Abstract

The use of green plants to remediate contaminated land (phytoremediation) has been proposed as a sustainable and cost-effective technique. Plants have been shown to have the potential to remediate land contaminated by organic and inorganic contaminants. The work presented here focuses on the potential for phytoremediation of land contaminated with the metals cadmium, chromium, copper, lead, nickel, and zinc.

The consideration of herbaceous plants which have the potential to produce a marketable end product as phytoremediation crops, has been limited. In this work the non-food species *Linum usitatissimum* (flax), *Brassica napus* var. *oleifera* (oilseed rape), *Miscanthus* × *giganteus* (miscanthus) and *Urtica dioica* (nettle) were investigated to assess their potential as phytoremediation crops.

Germination experiments using flax and oilseed rape established that seedling germination was not inhibited by exposure to metals in solution except at the highest concentrations considered. Germination was, however, not a reliable indicator of plant metal tolerance as metal toxicity to emerged seedlings was evident in contaminated soil treatments exhibiting good germination rates.

Four plant species were grown in soils containing six metals at both highly and marginally spiked levels, to reproduce genuine contaminated soils whilst allowing the study of each metal in isolation. A sewage sludge treated soil with a high metal and organic matter content was also included in the study. Miscanthus was the species most tolerant of the highly contaminated soils. The highest tissue concentrations recorded in plants exposed to the highly contaminated soils were (969 mg Zn/kg) in stems of miscanthus and (919 mg Cd/kg) in stems of nettle, but plant growth in these soils was generally poor. The plant species survived well in the sewage sludge soil, although metal uptake from this matrix was low.

Oilseed rape and nettle accumulated the highest tissue metal concentrations in the study of marginally contaminated soils. Indeed the highest tissue concentration recorded for plants grown in all of the soils was found in nettle grown in the

marginally contaminated Zn soils (1937 μg/g). Miscanthus, was able to remove a greater weight of metal from the soil owing to its higher biomass, despite having a lower tissue metal concentration than the other species.

The survival and growth response of flax to metals as well as metal uptake to flax tissues were studied in greater depth using hydroponic growth techniques. The upper solution concentrations of the six metals required to cause plant death were investigated using a nutrient film technique (NFT). Twelve varieties of flax assessed in a static hydroponic system indicated that there were only minor varietal differences in metal tolerance and metal uptake for the six metals at the concentrations investigated, however, Viola was the variety able to take up high metal tissue concentration relative to the other varieties consistently across all metals.

The manipulation of plant uptake response to metals in solution using both buthionine sulphoxamine(BSO) and histidine was investigated. Phytochelatins are a class of plant peptides implicated in detoxification of Cd in plant cells. The effect of buthionine sulphoximine, a chemical known to inhibit phytochelatin production, on flax plants grown in Cd-containing nutrient solution was investigated. The combined presence of BSO and Cd had no effect on plant yield compared to plants exposed to Cd alone, but did cause a reduction in root tissue Cd concentration. The response of flax to Cu and Ni solutions in the presence of histidine was also investigated. Histidine, which has been shown to protect plants from the toxic effect of Ni and increase Ni movement from roots to shoots, did not reduce the toxic effect of Cu on plant yield or of Ni on shoot yield. The presence of histidine reduced the toxic effect of Ni on root yield and lowered root Ni concentration. The simultaneous presence of histidine and Cu in nutrient solution increased flax root tissue Cu concentration and reduced shoot tissue Cu concentration.

Flax, miscanthus, nettle and oilseed rape have been shown to have potential to act as part of a phytoremediation programme, however, more work with these crops is required before firm advice can be given on commercial application of the crops in contaminated land remediation.

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Declaration

I hereby declare that this thesis was composed by myself and the work contained herein to be my own work, except where otherwise indicated.

John Kerr

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Abbreviations

Accum.	Accumulation
Ala	Alanine
BSO	Buthionine sulphoximine
Cd	Cadmium
Cr	Chromium
Ctrl.	Control
Cu	Copper
Cys	L-Cysteine
Glu	L-Glutamic acid
Gly	Glycine
GS	Glutathione synthase
GSH	Glutathione
His	Histidine
hPC	Homo-phytochelatin
HMT1	Heavy metal tolerance protein
ICRCL	Inter-departmental Committee on the Redevelopment of Contaminated Land (ICRCL)
Misc.	Miscanthus
MT	Metalothionines
NFT	Nutrient film technique
Ni	Nickel
OSR	Oilseed rape
Pb	Lead
PC	Phytochelatin
PCS genes	Phytochelatin synthesis genes
SE of mean	Standard error of mean
SS	Sewage sludge
st. dev.	Standard deviation
Zn	Zinc

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1 Introduction

The removal of the legacy of pollution that has been inherited from the industrial activities of the past, as well as from present pollution sources, is an important aspiration of today's society. Remediation of such sites is desirable from an economic, environmental and human health point of view. Traditional remediation strategies which normally involve "dig and dump" or encapsulation techniques are expensive and rather than addressing the problem of removing contaminants, simply transport the contaminants to another location or cover them up. Phytoremediation differs from traditional remediation strategies by its very nature. Phytoremediation is both sustainable, using renewable green plants, and systematic, conceptualising the pollution problem as part of a soil–plant system. Phytoremediation also aims to remove pollutants from the contaminated matrix into plant biomass, or in the case of organic pollutants by transformation to a less toxic form.

1.1 Contaminated land

1.1.1 Sources of contaminant metals in the environment

The most important sources of contaminant metals include fly ash, plastics, textiles, microelectronics, refineries and sewage sludge (Alloway, 1995; Nriagu and Pacyna, 1988). Several of the resulting contaminants are Cd, Cr, Cu, Pb, Ni and Zn.

1.1.2 Contaminated land in Scotland

A survey of Scottish Vacant and Derelict land was carried out in 2000 by the Scottish Executive (Scottish Executive, 2001). Vacant and derelict land were not necessarily contaminated nor was land in either category necessarily free from contamination, although where contamination was known or suspected the land was classified as derelict. Derelict land was defined by the Scottish Executive as follows:

> Derelict land (or buildings) is that which has been so damaged by development or use that it is incapable of being developed for beneficial use without rehabilitation, and which is not being used for the purpose for which it is held or for a use acceptable to a local plan.

The Vacant and Derelict land survey found that 11,683 ha of land in Scotland was either vacant or derelict in 2000, the greater part of which was derelict (7,432 ha). This area is less than 0.15% of the total land area of Scotland, however, the majority of these sites are in or near urban areas, situated mainly in the central belt. Major former uses of derelict land included 'mineral activity' (37%) and manufacturing (20%). Of the 1,622 ha of derelict land known to be contaminated, 10 ha were contaminated with Cr and 4 ha were contaminated with either Cu, Ni or Zn. Whilst the single largest known contaminant of derelict land was coal (539 ha), 682 ha were either unknown or listed as 'other contaminants'.

The true extent of land contaminated with metals is likely to have been underestimated since contamination has only been confirmed for sites where metal analysis has been conducted. The relatively small area of land in Scotland reported to be contaminated by metals does not reflect the impact such sites may have on human health and the environment. The location of contaminated land in predominantly urban areas increases the potential exposure of local populations to contaminants and poses problems for the redevelopment of otherwise geographically attractive sites. The former Ravenscriag steelworks site is a prime example of a contaminated site within an urban area requiring remediation and redevelopment (Scottish Enterprise, 2001).

The juxtaposition of contaminated sites to areas earmarked for economic regeneration can put a serious barrier on the redevelopment of an area. The high costs of conventional remediation techniques can often inhibit redevelopment of a site, and thus a lower cost option such as phytoremediation would be beneficial. However, phytoremediation is a long term remediation technique, compared with conventional remediation techniques, and requires financial input over the medium to long term. A phytoremediation technique which may be self financing or at least part funding, through the growing of crops which have a market value, is therefore an attractive concept.

1.2 Phytoremediation

Phytoremediation, from the Greek phyto (plant) and Latin *remedium* (to correct or remove evil), is defined as the use of green plants to remove pollutants from the environment or to render them harmless (Raskin *et al.*, 1994). Plants act like a wick, with a high surface area in the soil for collection of the soil solution and its soluble contents. This 'wick' is coupled to an efficient plumbing system driven by evaporation of water from the stomata, leaving non volatile soil solution components in the plant tissues where they can be removed by harvesting the plant. This system is an engineering solution to the problem of extracting soluble species intimately associated with soil perfected by 400 million years of evolution (Campbell, 1990).

The concept of utilising plants to decontaminate anthropogenic waste is very old; indeed secondary treatment of municipal sewage using plant based systems was recorded over 300 years ago and plants have been used for this purpose ever since (Cunningham *et al.*, 1996). Recently there has been renewed interest in this type of technology, including a garden designed to treat the sewage from a typical household exhibited in the 2002 Chelsea Flower Show (Royal Horticultural Society, 2002). Current interest in phytoremediation, however, extends beyond the treatment of sewage.

Hung *et al.* (1997) stated that the aim of phytoremediation is to reduce the Pb content of contaminated sites to acceptable levels in 3–20 years. This strict definition of phytoremediation is useful in highlighting the time scale for remediation. However, other authors have used the less constrained definition of phytoremediation proposed by Raskin *et al.* (1994), allowing the possibility of a longer term approach as well as the use of plants to stabilise rather than reduce the site contamination.

1.2.1 Phytoremediation techniques

Phytoremediation has been further subdivided into several distinct techniques: phytoextraction, phytodegradation, phytostabilization, phytovolatilization and rhizofiltration (Salt *et al.*, 1998). With the exception of phytodegredation, each of these techniques can be used to remediate land contaminated with heavy metals. Phytodegredation is limited to the remediation of organic pollutants which can be biologically metabolised to an acceptable end point. This is accomplished using the metabolic processes of both green plants and the micro-organisms associated with the rhizosphere of the green canopy (Burken and Schnoor, 1997; Cunninghamm *et al.*, 1996).

The remaining techniques use a variety of strategies to remediate both organic and inorganic pollutants. Phytoextraction is the use of green plants to extract pollutants from soils and sequester them in harvestable plant tissues (Kumar *et al.*, 1995). Phytostabilization uses the ability of green plants and their associated micro-organisms to transform pollutants to a form with a lower bioavailability to the environment (Dushenkov *et al.*, 1995). The definition of phytostabilization can be broadened to include the use of plants to prevent the physical movement of contaminants from contaminated sites such as reducing dust migration or the leaching of contaminants by establishing a green cover (Stomp *et al.*, 1993; Vangronsveld *et al.*, 1995). Phytovolatilization is the use of plants to promote the transfer of volatile species from the contaminated matrix to the atmosphere. Phytovolatization is the only phytoremediation technique which is a dilute and disperse mechanism (Burken and Schnoor, 1998; Schnabel *et al.*, 1997). Rhizofiltration is the use of plant roots to remove contaminants from water courses (Dushenkov *et al.*, 1995).

Continuous and chelate–assisted phytoextraction

Salt *et al.* (1998) further defined phytoextraction of metals into two strategies. The first of these, continuous phytoextraction, is the use of green plants which are both tolerant of, and able to accumulate, the contaminant metals for remediation. This strategy relies on plants removing metals from the soil into their above ground tissues steadily over the growing season. The plants initially proposed for this strategy were primarily metal hyperaccumulating plants (Baker *et al.*, 1988; Reeves *et al.*, 1996) and more recently high biomass species (Blaylock *et al.*, 1997, Robinson *et al.*, 1997). The second strategy reviewed by Salt *et al.* (1998), they termed induced phytoextraction or chelate-assisted phytoextraction. This strategy differs from the continuous phytoextraction in that the plants grown in the soil need not accumulate the metals in their tissues steadily over the growing

season. Instead, plants that are tolerant of the soil metals and able to produce a high harvestable biomass can be grown in contaminated soil. When the plants reach an optimum biomass, a chelating agent is added to the soil. The chelating agent mobilises the contaminant metal(s) by desorption from solid phase soil constituents into the soil solution, the soluble chelated metals can then be drawn into the harvestable plant tissues by mass flow in the transpiration stream. Thus metals are removed from the soil and out of the soil system upon harvest of the crop biomass.

1.2.2 Current trends in phytoremediation research

Phytoremediation has been extensively reviewed (Black, 1999; Chaney *et al.*, 1997; Cunningham *et al.*, 1995; Flathman and Lanza, 1998; Glass, 1998; Raskin, 1996; Raskin *et al.*, 1997; Salt *et al.*, 1998). Interest in the identification and characterisation of metal hyperaccumulating plants largely growing on naturally metaliferous or serpentine soils (Baker *et al.*, 1988; Reeves *et al.*, 1996) has more recently been augmented by the study of high biomass crops with similar characteristics to the metal hyperaccumulating crops, often in related plant families (Kumar *et al.*, 1995). Several researchers have investigated the use of trees for phytoremediation, in particular willow short rotation coppice, reviewed by Pulford and Watson (2002). There has, however, been limited study of other high-biomass crops and existing non-food crops for phytoremediation.

1.2.2.1 Prospects for the use of transgenic phytoremediator crops

The existence of the biochemical mechanisms for metal hyperaccumulation in combination with the emerging technologies of genomics and of genetic manipulation has the potential to enable plants with high biomass and metal accumulating traits to be engineered. The use of biotechnology to engineer an ideal phytoremediation crop has been highlighted (Ow, 1996; Salt *et al.*, 1998; Stomp *et al.*, 1993; Zhu *et al.*, 1999). The co-occurrence of both high biomass crops, such as Indian mustard, and hyperaccumulating plants, such as *Thlaspi caerulescens*, in the genus *Brassicaceae* may facilitate efforts to engineer such plants. Gleba *et al.* (1999) were able to produce a somatic hybrid of the hyperaccumulator species *Thalaspi caerulescens* with the high biomass species *Brassica juncea* which extracted more lead from the soil than either of its parents. This technology will, however, be limited to remediating soils contaminated with metals for which accumulating traits have evolved. There are important contaminants for which few accumulating plants have been reported, including lead (Salt *et al.*, 1998), although recent reports of lead accumulating species indicate that plant uptake mechanisms may exist allowing remediation of a wider range of metals than was first proposed (He *et al.*, 2002; Sahi *et al.*, 2002).

Genetic modification using both phytochelatins (PCs) and metalothionines (MTs) to increase plant tolerance to Cd has been investigated. Zhu and colleagues (1999) showed that the use of genetic modification can improve plant tolerance to Cd and increase Cd removal from the rhizosphere to the harvestable portion of the crop. They reported that transgenic *Brassica juncea* plants over expressing a glutathione synthase protein (*Eshcerichia coli gshII* gene) had a greater biomass production and 40% greater shoot Cd concentration, than the wild type plants. In contrast, Maiti and colleagues (1989) reported that *Brassica napus* and tobacco, genetically modified to express mouse MT genes, also gave an increased tolerance to Cd, however this was accompanied by reduced Cd concentration in the leaves.

1.2.3 Hyperaccumulators and continuous phytoextraction

The ability of plants to accumulate high levels of heavy metals is not a new concept. In 1885, Baumann reported atypically elevated levels of zinc in plants growing on naturally zinc enriched soils. More recently a model of continuous phytoextraction using metal hyperaccumulating plants has been introduced by Chaney (1983) and supported by Baker *et al.* (1988). Continuous phytoextraction of sites contaminated with metals such as Ni and Zn is proposed using plant species endemic to naturally occurring metaliferous soils which have evolved the ability to accumulate the metals in their tissues at unusually high levels (Salt *et al.*, 1998). Many of these plant have the ability to accumulate a tissue metal concentration greater than the metal concentration of the surrounding soil and are therefore known as hyperaccumulators.

Reasons for plant uptake

Plants which have the ability to grow on soils with high heavy metal concentrations can exploit an ecotype unavailable to other species and thereby encounter reduced competition. This explains the evolution of metal tolerant ecotypes, however, hyperaccumulator species must gain some additional advantage in having increased tissue metal concentrations. Boyd and Martens (1994) found that the leaf tissue of the hyperaccumulator *Thlaspi montanum* contained 3000 μg/g Ni and was acutely toxic to cabbage white butterfly larvae. The presence of Ni-containing sap was demonstrated to deter insect predation of *Sebertia acuminata* using Drosophila (Sagner *et al.*, 1998). The brassica, *Serptanthus polygaloides*, which was found to accumulate Ni, gave protection against pathogens: biotrophic fungus (powdery mildew, *Erysiphe polygoni*), bacteria (*Xanthomonas campestris* pv. *Campestris*) and prevented necrotic fungus *Alternaria brassicicola* growing on detached leaves or wound sites (Boyd *et al.*, 1994). These predator- and disease-inhibiting effects of the metal content of the hyperaccumulator plants would appear to explain their evolution. Gabbrielli *et al.* (1991) speculated that a high concentration of Ni in the leaves of *Alyssum bertolonii* and the subsequent shedding of leaves lead to further concentration of Ni in the soil layer immediately surrounding the plant, making the soil matrix yet more inhospitable for potential competitors.

More than 80 hyperaccumulators (defined as those plants which were able to accumulate tissue concentrations >1000 μg/g Ni) growing on serpentine soils in Cuba have been identified (Reeves *et al.*, 1996). The serpentine soils of Cuba were thought to have a particularly large number of hyperaccumulators due to the length of time serpentine soils have been continuously supporting plant populations.

Plants growing on serpentine soils fall into two groups: those with metal tolerating strategies which exclude metal from their tissues and those which accumulate metal in their tissues (Reeves *et al.*, 1996). Gabbrielli *et al.* (1990) studied two species, *Silene italica* and *Alyssum bertolonii*, that had evolved a Ni-excluding and Ni-tolerating strategy, respectively. When exposed to 7.5 μM Ni, *S. italica* exhibited root growth inhibition accompanied by an increase in peroxidase activity and phenol concentration in both root and shoot tissue, but *A. bertolonii* did not exhibit any of these responses. Gabbrielli and colleagues (1990) also reported that *A. bertolonii* accumulated Ni at greater concentrations in shoot tissue than root tissue, whereas *S. italica* held Ni predominantly in root tissue. Nickel accumulated in root and shoot tissue of *A. bertolonii* was associated with malic acid and this may be linked with Ni tolerance (Gabbrielli *et al.*, 1991).

To establish whether the roots of a hyperaccumulator had a greater ability to acidify or reduce the surrounding soil matrix than a non-accumulator, Bernal *et al.* (1994) compared the accumulator plant *Alyssum murale*, with the non-accumulating crop plant *Raphanus sativus* (radish). *Alyssum murale* did not excrete more protons or reductants than *R. sativus* indicating the hyperaccumulator had another strategy for mobilising soil Ni, likely by secretion of root exudates. Krämer *et al.* (1996) reported that exposure of *Alyssum lesbiacum* (an accumulator species) to 0.3 mM Ni resulted in a 36-fold increase in the amino acid L-histidine in xylem sap. Non-accumulator species, *A. montanum*, did not exhibit any change in amino acid content in the xylem sap. The hyperaccumulator species *Assylum lesbiacum*, *Alyssum murale*, and *Alyssum bertolonii* were all observed to have a linear relationship between xylem Ni and histidine concentrations. The authors also supplied histidine to the non-tolerant *A. montanum* species and found that, in the presence of Ni, biomass production was doubled and xylem transport of Ni was increased. Krämer *et al.* (1996) concluded that histidine was important in affording hyperaccumulating *Assylum* species their tolerance to Ni and allowing Ni to be transported in the xylem to the shoot.

Thlaspi caerulescens has been found to accumulate the metals: Ag, Al, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn (Baker *et al.*, 1994). Some metals were readily transported to the shoots (Zn, Cd and Ni) whereas others were held predominantly in the roots (Cr, Cu and Pb). A study of *Thlaspi caerulescens* in solution culture revealed a greater accumulation of metal than was observed for plants grown in metal-containing soils, with metal bioaccumulation in plant tissue between 300 and 2000 times the solution concentration (Baker *et al.*, 1994). The metals were accumulated from solution in increasing quantities in the order: Pb, Cr, Ni, Zn, Cd and Cu. Roots held 10–100 times more metal than shoots for Cr, Cu and Pb. For Zn and Ni, more metal was held in the shoots than the roots and the root:shoot ratio for Cd was ~ 1. Baker *et al.* (1994) speculated that *Thlaspi caerulescens* has a constitutive metal tolerance mechanism which allows the accumulation of a wide range of metals.

Brown *et al.* (1995a) also observed *Thlaspi caerulescens* to be an effective hyperaccumulator of both Cd and Zn and proposed that it may be a suitable species for phytoremediation. However, in a study comparing *Thlaspi caerulescens* with *Silene vulgaris* and cos lettuce grown in a long term sewage sludge plots, it was found that *T. caerulescens* was not any more efficient in removing Cd than *S. vulgaris* or lettuce (Brown *et al.*, 1995b). Furthermore, the growth habit of *T. caerulescens* was less appropriate for phytoremediation than the other plants (Brown *et al.*, 1995b). Krämer *et al.* (1997) reported that *Thlaspi goesingense*, a hyperaccumulator of Ni was able to accumulate high concentrations of Ni in its tissues as a result of an enhanced tolerance mechanism in the protoplast rather than as a result of enhanced transport of Ni. Investigations of a Ni-accumulating tree, *Sebertia acuminata*, revealed a latex Ni content of 15–30% and have shown that Ni was predominantly localised in phloemic laticifers where it was associated with citrate and possibly nitrate anions (Sagner *et al.*, 1998).

1.2.4 High biomass crops for phytoremediation

Most hyperaccumulators have a low biomass and/or slow growth rates and so are inefficient plants for phytoextraction (Salt *et al.*, 1998; Gleba *et al.*, 1999). For this reason the identification of fast growing high biomass plant species able to accumulate contaminant metals has been undertaken (Kumar *et al.*, 1995; Pulford and Watson, 2002; Robinson *et al.*, 1997; Wilkins, 1997). These plants may allow a more efficient form of continuous phytoextraction with the possibility of generating revenue through use commercial exploitation of the plant biomass.

Tissue concentrations have been reported for high biomass plant species. Metal tissue concentrations of willow clones have been measured as: 3–76 μg Cd/g, 4–25 μg Cu/g, 17–157 μg Pb/g, 3–27 μg Ni/g and 77–702 μg Zn/g (Pulford *et al.*, 2002; Punshon and Dickinson, 1997a; Riddell-Black, 1994). Another high biomass crop, *Berkheya coddii*, was found able to accumulate a Ni concentration of 1% as well as a biomass of 20 t/ha (Robinson *et al.*, 1997). Shoot tissue Pb concentrations for *Brassica juncea* grown in contaminated soil (2500 mg/kg) ranged from 30–129 μg/g. However, Pb accumulation in maize shoots has been reported at the higher concentration of 225 μg/g (Huang and Cunningham, 1996).

A number of researchers have studied *Brassica juncea*. Kumar *et al.* (1995) found *B. juncea* had the remarkable ability to transport Pb from roots to shoots particularly at high concentrations although the roots still had 5-fold more Pb than shoots. *Brassica juncea* had five times more Cd in roots than shoots and more Cd in both roots and shoots than the accumulator plant *T. caerulescens* (Salt *et al.*, 1995). Gleba *et al.* (1999) found *B. juncea* combined a high shoot biomass with an ability to absorb EDTA–chelated Pb from contaminated soils and transport it to the shoots. *B. juncea* can produce a biomass of 18 t/ha although these yields will probably not be possible in contaminated soils (Blaylock *et al.*, 1997). Huang *et al.* (1996) found that *Zea mays*, a perennial C4 grass, extracted more Pb from both hydroponic culture and soil than *B. juncea*, and the hyperaccumulator *T. calurescenes*, *T. rotundifolium* and *Ambrosia artemislifolia*.

The use of trees with rapid growth habits to remove metals from contaminated sites has been proposed as an alternative strategy for phytoremediation to the use of hyperaccumulators. In particular trees managed by short rotation coppicing including willow and alder have been studied (Pulford and Watson, 2002). Trees like other high

biomass crops compensate for the lower tissue metal concentrations found in their tissues by virtue of the quantity of plant tissue produced per m^2 of contaminated land.

1.2.5 Chelate-assisted phytoextraction

Chelate-assisted phytoextraction has been proposed as a strategy for the remediation of contaminant metals for which there are few or no identified hyperaccumulating plants. The lack of identified natural plant adaptations to hyperaccumulate some metals means that the rapid removal of soil contaminants, possible for metals such as Zn and Ni using hyperaccumulating plants, or transgenic plants produced using their genes, is not possible. This chelate-assisted phytoremediation method has been studied particularly with relation to Pb. Chelate-assisted phytoremediation uses the ability of high biomass crops to provide a substantial weight of plant tissue to act as a sink for sequestered metals.

Chelate-assisted phytoextraction has two distinct stages: upon addition to the soil the chelating agent forms a metal–chelate complex with the metal in the soil thus mobilising the metal to the free soil solution; after mobilisation in the soil solution the metal–chelate complex is taken up by the plant roots and transported to above ground plant tissues (Salt *et al.*, 1998).

Using EDTA for chelate-assisted phytoextraction shoot tissue, concentrations of 1471 μg/g have been achieved using *B. juncea* and it has also been found that metal uptake by *B. juncea* is proportional to the affinity of the chelating agent to the metal in question (Blaylock *et al.*, 1997). Investigation of the effect of addition of HEDTA to the soil established that *Zea mays* shoot Pb concentration increased from 40 mg/kg to 10600 mg/kg in maize (Huang *et al.*, 1996). Increased removal of Pb from the roots as found upon addition of chelating agents (Huang *et al.*, 1996) and acidification of the soil matrix (Blaylock *et al.*, 1997) could enhance the performance of perennial crops such as miscanthus and willow since the root tissues would have lower metal concentration and therefore have a lower risk of suffering metal toxicity in their unharvested perennial tissues. For Pb, removal rates could be as much as 180–530 kg/ha per year with the addition of appropriate chelating agents (Blaylock *et al.*, 1997; Huang *et al.*, 1997).

1.2.6 Mechanisims for metal uptake

Heavy metal hyperaccumulating plants require the ability to withstand tissue metal concentrations other plants would find fatally toxic. There are several mechanisms proposed for the tolerance of different plants to different metals, these include: chelation, compartmentalisation, biotransformation (the chemical reduction of metalloids e.g. Cr, Se and As or their incorporation into organic molecules) and cellular repair mechanisms (Salt *et al.*, 1998).

1.2.6.1 Phytochelatins

A group of short-chained peptides synthesised by green plants has been implicated in metabolic mechanisms for plant–metal tolerance; these peptides have been given the name phytochelatins (PCs) and homo-phytochelatins (hPCs). Phytochelatins have the structure $(\gamma\text{-Glu–Cys})_n\text{–Gly}$ (n=2–11) whilst homo-phytochelatins have the structure $(\gamma\text{-Glu–Cys})_n\text{–}\beta\text{-Ala}$ (n=2–7) (Grill *et al.*, 1985; Rauser, 1990; Zenk, 1996). The biochemical role of PCs has been investigated and their function in regulating metal homeostasis in cells is analogous to MTs. Mammalian MTs contain 61 amino-acid residues and therefore are much bigger than PCs which, with n=2–11, have 5–23 amino acids (Grill *et al.*, 1985). The PCs also differ from animal metalothionines (MTs) in that the recurrent γ-carboxyamide is not known to be synthesised by ribosomes and so, unlike animal MTs, PCs are not primary gene products (Grill *et al.*, 1985; Rauser, 1990). Phytochelatins have therefore been shown to differ both structurally and in their synthesis from animal MTs (1 and 2). Class 1 and 2 MTs have been reported in metal tolerant ecotypes of *Arabidopsis thaliana* (Murphy and Taiz, 1995; Murphy *et al.*, 1997).

Cadmium is the metal species which has been most frequently linked with PC production and complexation, although other metals have also been shown to induce PC production and form complexes with PCs (Rauser, 1990). Phytochelatin synthesis has been reported in the presence of Cd^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+} and also to a lesser extent Pb^{2+}, Sb^{3+} and Ni^{2+} (Grill *et al.*, 1985). In a survey of the plant kingdom, all plants investigated synthesised PCs or h-PCs in response to Cd exposure (Gekeler *et al.*, 1989). The study included over 200 plants with representatives from both gymnospermae and angiospermae (including both dicotyledons and monocotyledons). They also found that the homo-phytochelatins were associated exclusively with some members of the angiosperm order Fabales (Leguminosae) which produce homo-glutathione rather than glutathione. Whilst PCs have been found in organisms outside the kingdom Phyta, notably in some fungi, their

ubiquitous occurrence in members of the kingdom Phyta seem to be sufficient justification for the name phytochelatin (Gekeler *et al.*, 1989).

Since the ability to synthesise PCs is ubiquitous in plant species they have been proposed as bio-markers for potential human health problems as their presence is indicative of heavy metal stress and, therefore, the potential presence of hazardous metals within the plant derived foodstuffs (Keltjens and van Beuichem, 1998).

Clemens *et al.* (1999) isolated a family of genes mediating PC production called PCS (phytochelatin synthesis) genes. PCS genes were shown to mediate PC production and play a central role in Cd tolerance. When the PCS genes were deleted, PCs were notsynthesised and plants were no longer tolerant to Cd. Inclusion of the PCS genes in an organism with no PCS homologue resulted in the organism gaining Cd tolerance.

Phytochelatins detoxify metals by forming co-ordinate (mercaptide) complexes with the metal ions via the thiolate groups of cysteine (Grill *et al.*, 1985). It is proposed that PCs reduce metal toxicity within the plant cell by preventing metal sulfohydryl group interference by sequestering free metal from the cytosol, allowing normal cellular metabolism (Rauser, 1990). Clemens *et al.* (1999) observed that Cd detoxification was not solely a function of PC synthesis, but PCs gave the plant the capacity to buffer the metal in the cytosol prior to sequestration in the vacuole. Phytochelatins can reactivate Cd inactivated enzymes more effectively than other chelating agents, GSH or citrate, due to their high affinity for Cd (Kneer and Zenk, 1992). Grill *et al.* (1987) showed that exposure to Cu arrested growth of *Rauvolfia serpentinia* cells for a 10-h period after which PCs were synthesised. After two days, 80% of the Cu had been removed from the culture medium and normal cell growth had resumed giving evidence for PC conferred detoxification of Cu.

Parallels have been drawn between PCs and phytosiderophores as well as exogenous chelating agents such as humic acids citrate or EDTA as they are all chelating agents which provide plant cells with balanced metal homeostasis to allow optimum function of cellular processes for plant yield maximisation (Kinnersley, 1993).

1.2.6.2 Buthionine sulphoximine (BSO)

BSO is a powerful and specific inhibitor of the glutathione biosynthesis pathway (Reese and Wagner, 1987). Cadmium-tolerant plants exposed simultaneously to Cd and BSO showed growth inhibition, however, the plants were unaffected by exposure to BSO in the absence of Cd (Rauser, 1990). Moreover, when glutathione was supplied artificially to the plant cells, PC biosynthesis proceeded in the presence of BSO showing that glutathione synthesis is a necessary precursor of PC synthesis (Rauser, 1990).

Gussarsson *et al.* (1996) found that birch exposed to both Cd and BSO suffered a detrimental change in nutrient status, inhibition of growth of both roots and shoots, and that Cd accumulation in all parts of the plant was reduced in the BSO treated plants. Krotz *et al.* (1989) indicated that PCs protect cells against metal-induced damage by removing free metal from solution but that compartmentalisation of Cd in the vacuole occurs irrespective of the presence of PCs.

1.2.6.3 PC synthesis as a response to metal stress

Since the discovery and characterisation of PCs as metal-binding peptides the synthesis of PCs has been proposed as a mechanism for metal tolerance in plants. Several authors, however, have argued that the synthesis of PCs is a response to metal-induced stress and their presence does not in itself confer metal tolerance to plant species able to synthesis PCs.

Although heavy metal tolerance in some cells has been correlated with rapid and early formation of Cd-PC complexes, not all plants exhibit metal tolerance via PC synthesis (Rauser, 1990). Although PC synthesis has been shown to be ubiquitous in the plant kingdom (Gekeler *et al.*, 1989), relatively few plants have been shown to be tolerant of excessive cell loadings of heavy metals. Phytochelatin production does not, therefore, necessarily confer heavy metal tolerance to plants (de Knecht *et al.*, 1992; de Knecht *et al.*, 1994; Schat and Klaff, 1992). It has also been reported that PC production was induced in the presence of heavy metal in both tolerant and non-tolerant plants (Shat and Klaff, 1992), however, in tolerant plants, PC synthesis took place at higher metal concentrations than in non-tolerant plants, suggesting that the presence of PCs was an indication of tolerance independent metal stress. Comparison of Cd-tolerant and Cd-sensitive *Silene vulgaris* plants established that upon exposure to Cd, both plants produced PCs (de Knecht *et al.*, 1992). Although the Cd-sensitive plants produced more PC than the Cd-tolerant plants, the Cd-sensitive plants had twice as much Cd in their root tissue compared to the Cd-tolerant

plants. Phytochelatin production was inhibited in both the Cd-sensitive and Cd-tolerant plants when exposed simultaneously to BSO and Cd, however, this only affected the growth of the Cd-sensitive plants. Cadmium-tolerant plants produced lower rates of PC than Cd-sensitive plants whilst the PC chain length in Cd-tolerant plants was longer than that of Cd-sensitive plants (de Knecht *et al.,* 1994). Differential tolerance in *Silene vulgaris* may be the result of faster transport of Cd from the cytoplasm to the vacuole, thus reducing the cytosol Cd concentration and consequently PC production (de Knecht *et al.,* 1994). Whilst PC production may contribute to the mechanism of Cd detoxification, the ability of plants to produce PC in higher quantities does not confer differential metal tolerance. These reports imply that metal tolerant plants have additional or more efficient detoxification mechanisms compared to non-tolerant plans, and that these mechanisms do not necessarily involve PC production.

1.2.6.4 Root exudates

An ideal phytoextractor crop must have the ability to tolerate, solubilise and subsequently remove toxic metals from the soil. Plants must be able to extract essential nutrient elements from soils and consequently they have evolved mechanisms to exploit the reservoirs of metal ions not normally available in the soil solution. Three important root induced metal solubilising processes have been identified: changes of pH in the rhizosphere, increased reducing capacity of the roots and synthesis of root exudates (Bernal *et al.,* 1994).

Root exudates, known as phytosiderophores, can increase metal solution concentration in soil intimately associated with root tissue, facilitating efficient removal of metals from the rhizosphere. Phytosiderophore compounds identified include mugenic acid and avenic acid (Kinnersley, 1993).

Phytosiderophores are normally synthesised in response to a deficiency of essential nutrients and have been well documented in mediating the uptake of Fe in Fe-deficient soils (Higuchi *et al.*, 1994). Phytosiderophores are also known to chelate Cu, Zn and Mn (Romheld, 1991). The phytosiderophores form soluble metal–chelate complexes, mobilising metals from the solid phase to the soil solution from where they are transported across the root plasma membrane by specialised transporters (Von Wirén *et al.*, 1996). A Fe–phytosiderophore membrane transport protein, in maize, was reported to also transport Zn–phytosiderophore complexes and that the transport of free Zn was also observed (Von Wirén *et al.*, 1996). Zinc efficient maize ecotypes with enhanced phytosiderophore synthesis and enhanced phytosiderophore-mediated Zn transport have also been observed

to increase the mobility of Zn, both from the rhizosphere to the plants, and within the plant (Cakmak *et al.*, 1996). Both Cakmak *et al.* (1996) and Von Wirén *et al.* (1996) studied maize responses to Zn deficiency, rather than the higher levels of Zn present in Zn contaminated soils, however, the existence of mechanisms for phytosiderophore production and transport present the opportunity for exploitation of such traits by genetic engineering.

In a study of *B. juncea* grown in metal-containing nutrient solution, significantly more Cd, Cr, Cu, Zn and Ni was removed from solution than was recovered in root tissue, an observation speculated to be attributable to metal precipitation by root exudates in the solution (Dushenkov *et al.*, 1995). Dushenkov *et al.* (1995) also found that lead was precipitated in the roots mainly as lead phosphate.

In addition to the use of phytosiderophores, plants can also use reductase enzymes in the rhizosphere to release elements in their soluble form; alternatively, plants can pump protons from their roots acidifying the rhizosphere to solubilise acid soluble elements (Crowley *et al.*, 1991).

1.2.6.5 Non-PC detoxification mechanisms

Detoxification mechanisms which do not involve PCs have been identified. Murphy and Taiz (1995) reported that Cu-tolerant ecotypes of *Arabidopsis thaliana* synthesised both MT1 and MT2; in roots MT1 was synthesised constitutively and MT2 synthesis was induced by Cu stress, whereas in leaf tissue, MT1 synthesis was induced by Cu stress whilst MT2 was synthesised constitutively. Furthermore, Cd sensitive *Arabidopsis thaliana*, which lacks an ability to synthesis PCs, showed little reduction in Cu or Zn tolerance, suggesting PCs were important for Cd tolerance but did not play an important role in Cu or Zn tolerance (Howden *et al.*, 1995; Murphy *et al.*, 1997).

Precipitation of Zn as zinc phytate in globular bodies within the vacuole has been observed and was proposed as a detoxification method in *Deschampsia caepitosa* (Van Steveninck *et al.*, 1987); these globular deposits have been located in parenchyma cells of *Lemna minor* (Van Steveninck *et al.*, 1990). Organic acids other than phytic acid have been implicated in metal tolerance strategies. Ni accumulation in tissues of ryegrass and maize was associated with citric and malic acid (Yang *et al.*, 1997) and both Cd and Zn can be complexed by organic acids and compartmentalised in the vacuole of tobacco cell-suspension culture (Krotz *et al.*, 1989). Organic acid levels in cell-suspension culture were

reported to exceed Cd and Zn levels, even at growth inhibiting Cu and Zn concentrations (600 μM and 2000 μM, respectively); malic acid was the predominant acid followed by oxalic acid and citric acid, all of which, in addition to Cd, were found predominantly isolated in the vacuole (Krotz *et al.*, 1989).

Salt *et al.* (1995) reported that Cd did not appear to be transported by PCs in xylem sap as Cd was found to interact with oxygen or nitrogen molecules. They speculated that these oxygen and nitrogen elements were probably present in organic acids. In an investigation of the transport of Ni in tissues of *Alyssum lesbiacum*, the principal organic acids (citrate and malate) did not increase in concentration in response to Ni exposure (Krämer *et al.*, 1996). The chelating agent identified as facilitating the transport and detoxification of Ni was histidine (Krämer *et al.*, 1996). Both Krämer *et al.* (1996) and Yang *et al.* (1997) found Ni was transported in the xylem. Transgenically enhanced plants tolerant to Cd have shown increased Cd translocation in the xylem transpiration stream (Zhu *et al.*, 1999). Other authors have reported metal transport in the xylem transpiration stream: Salt *et al.* (1995) found Cd accumulation to the shoots appeared to be driven by mass flow in the transpiration stream, although, Cd uptake to the roots was independent of transpiration rate whilst Blaylock *et al.* (1997) were able to show by inhibiting evapotranspiration that the transpiration stream was important in the translocation of Pb.

Plants can detoxify toxic metal species by biotransformation mechanisms which change the chemical properties of the metal species. This can be achieved by chemical reduction of the metal species to a less toxic form, for example Cr(VI) to Cr(III) (Dushenkov *et al.*, 1995). Other biotransformations include plant incorporation of metal species into organic molecules, thereby preventing them from taking part in other cellular reactions, as described for *Astagalus* species which accumulate Se. These plants are able to avoid Se toxicity by incorporating Se into the non-protein amino acids methylselenocysteine and selenocystathionine; channelling the toxic metal into these amino acids prevents the Se being incorporated into selenocysteine and selenomethionine, compounds which would otherwise disrupt protein function (Lauchli, 1993).

1.2.6.6 Compartmetalisation

Compartmentalisation at the whole plant level

Hyperaccumulator species differ from non-accumulator species in that they tend to have higher metal tissue concentrations in above ground tissues than in root tissues (Baker *et al.*, 1994; L'Huillier *et al.*, 1996), whereas non-accumulator crops tend to have metals

predominantly compartmentalised in roots (Pulford *et al.*, 2001; Punshon and Dickinson, 1997). Dicotyledonous plants have been found, independent of soil solution metal concentration, to accumulate up to 4-fold more Pb in their roots than monocotyledonous plants (Huang and Cunningham, 1996). It has been indicated that this may be due to dicotyledonous plants being more efficient in transporting Pb from the apoplastic stream into root cells via a voltage-gated Pb^{2+} (Ca^{2+}) transporter.

Compartmentalisation in plant tissues

Trichomes are hair-like projections from plant epidermal cells. Some trichomes have evolved specialist functions, for example, the stinging barbs of nettle plants and the polyphenol oxidase containing insect traps of solanum species (Yu *et al.*, 1992). In the leaf tissue, Cd was preferentially accumulated in the trichomes, resulting in a trichome Cd concentration of 556 μg/g, which was 43 times more concentrated than leaf tissue Cd concentration (Salt *et al.*, 1995). Trichomes are an external tissue which have been shown to accumulate metals in other plants: Mn in sunflower foliar trichomes (Blamey *et al.*, 1986), Cu in glandular trichomes of Solanum species (Kowalski *et al.*, 1992), and Pb in trichomes of tobacco (Martell, 1974). Metalothoinines have been found associated with foliar trichomes (Foley and Singh, 1994) and it has been speculated that MT synthesis is confined to specialist plant tissues such as trichomes (Murphy *et al.*, 1997).

Compartmentalisation at the sub-cellular level

An investigation of Ni transport in the tonoplast of oat roots found that the lack of transport mechanisms at the vacuole minimised Ni sequestration in the vacuole compared to Ca and Cd, therefore the vacuole was found not to be an important compartment for Ni in oat roots (Gries and Wagner, 1998). In the metalophyte *Thlaspi caerulescens,* which has been found to tolerate Cd and Zn, Cd was sequestered in the apoplast whilst Zn was found predominantly in the vacuole (Vasquez *et al.*, 1992). Cd was also found in the vacuole where it was associated with dense fibrous material but this storage site was less significant than the apoplast, similarly, Zn was found in the cell walls, but to a lesser extent than in the vacuole. These findings indicated that within plant species the preferred compartment for sequestration varies according to metal.

Ow (1996) using yeast *Schizosaccharomyces pombe* as a model system, reported that Cd storage capacity was increased by incorporation of sulphide in the vacuole. Cadmium in shoots of *Brassica juncea* was found to be bound with S-ligands possibly as Cd–S_4 (Salt *et al.*, 1995). The formation of Cd–S crystallites surrounded by PC peptides has been reported (Dameron *et al.*, 1989). The presence of these crystallites increased the ratio Cd:PC and also increased the stability of the Cd complex in the vacuole (Ow, 1996). Speiser *et al.* (1992) found that Cd forms a high molecular weight Cd–PC–Sulphide complex in cell free extracts from *Brassica juncea* seedlings. The high molecular weight Cd–PC–Sulphide complex was more stable than the low molecular weight PC-Cd complex formed in the cytoplasm and so may lead to higher metal tolerance through more efficient sequestration. Transgenic *Brassica juncea* plant, which overexpressed a glutathione synthase (GS) protein, also had greater sulphide concentrations than the wild type plants (Zhu *et al.*, 1999).

Vogeli-lang and Wagner (1990) reported that Cd was located in the vacuole of tobacco cells, associated with PCs and that the PC–Cd complex formed in the cytoplasm and was then transported into the vacuole. Speculation followed that PCs act as a shuttle system to carry Cd to the vacuole (Gussarsson *et al.*, 1996). A protein, named HMT1 (heavy metal tolerance), has been found to transport cytoplasmic PC–Cd complexes into the vacuole (Ow, 1996). Ow (1996) also found a Cd-induced sulphide generating pathway where the S^{2-} is derived via a novel synthetic pathway from cysteine and concluded that the sulphide generating pathway may be as important in sequestering high levels of Cd in the vacuole as the PC-Cd transport protein. This supported the argument that PC synthesis alone does not confer metal tolerance to plants.

Two mechanisms for sequestration of Cd from the cytoplasm to the vacuole have been identified. Salt and Wagner (1993) described a pH gradient-dependant Cd active transport system. This was shown to be a Cd^{2+}/H^{+} antiport driven by V-type ATPase generated pH gradient and was proposed as a mechanism for Cd^{2+} transport from the cytoplasm to the vacuole at both high and low Cd exposure. Salt and Rauser (1995) proposed that a Mg ATP-dependent transporter was responsible for the sequestration of both PC and Cd–PC from the cytosol across the tonoplast into the vacuole of oat root cells. They suggested this transporter belonged to the superfamily of ABC type transporters. The ATP-dependent transport mechanism was unaffected by pH gradient inhibition and so is in addition to the pH gradient driven Cd^{2+}/H^{+} antiport (Salt and Wagner, 1993).

1.3 Plants used in the study

Novel applications of crop for industry may provide appropriate end uses for phytoremediation crop biomass. Crops grown in contaminated land, with the explicit goal of concentrating heavy metals in their harvested tissues, may be unwelcome feedstocks for some processes and end uses but may be appropriate for others. In particular, the use of plant biomasss for energy production through combustion would be an ideal application as plants would have a marketable value and the combustion process would allow concentration and containment of the metals as ash. Biomass crops have been proposed as an alternative source of fuel for energy production (Grassi, 1999; Hunter, 1996; Speller, 1993; Wilkins and Abrutat, 1995).

1.3.1 Flax

The use of flax as a fibre crop has a long history as cloth woven from flax has been found in the tombs of Egypt (Grieve, 1931). *Linum usitatissimum* is commonly known as flax or linseed. Dahlke *et al.* (1998) identified natural fibres as being suitable for the production of automotive interiors. In particular the use of flax, hemp and sisal based automotive interior trims are cited as having similar, and in some cases, preferable properties to those of the glass fibre based products currently used. Natural fibres would be cost effective and environmentally friendly substitute for current materials. The application of flax fibres to the manufacture of composite materials is of current interest (Aurich and Mennig, 2001; Bos *et al.*, 2002; Hodzic *et al.*, 2002; Zafeiropoulos *et al.*, 2001).

When used for oil production, varieties *Linum usitatissimum* are known as linseed and when used for fibre production they are known as flax. These two groups of *Linum usitatissimum* varieties have developed morphologically differences, each having been bred to optimise yield of the desired end product. However, the use of dual purpose varieties of *Linum usitatissimum* for the simultaneous production of oil and fibre has been proposed to maximise returns from the crops, for example, the use of *Linum usitatissimum* for the production of oil and for fibre for textile with the residue processed to pulp for paper making (Shaikh *et al.*, 1992).

1.3.2 Miscanthus

Miscanthus × *giganteus* (miscanthus) is a perennial grass with woody stems which reach heights of 2–4 m. The stems senesce in autumn, but the plant is able to regenerate stems in the spring from its rhizomatus root system (Speller, 1993). *Miscanthus* × *giganteus* is an interspecific hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus* (Hodkinson *et al.*, 1997) which occurs naturally in sub-tropical areas of East Africa and Asia but has been successfully grown in the cool temperate climate of northern Europe (Beale and Long, 1995; Beale *et al.*, 1996; Bullard *et al.*, 1997; Christian *et al.*, 1997).

Miscanthus is proposed as a potential biomass crop to provide a carbon neutral feed stock for energy production (Bullard *et al.*, 1997; Christian *et al.*, 1997; Wilkins, 1997) Miscanthus foliage dies at the end of the growing season and so can be harvested at a high dry matter content giving the biomass favourable combustion properties (Speller, 1993). Other potential uses for miscanthus include geotextiles, building applications and paper pulp (Huisman *et al.*, 1997; Ellison and McNaught, 2000).

In common with maize, miscanthus has a C-4 photosynthetic pathway. Miscanthus has been demonstrated to achieve close to maximum light interception and conversion efficiency for C-4 plants which exceed those of C-3 crop plants traditionally grown in northern Europe (Beale and Long, 1995). Miscanthus has also been shown to be less sensitive to cold-induced growth reduction than maize (Beale *et al.*, 1996) indicating that miscanthus may be better suited to the UK climate than maize. Maize is currently grown commercially in the UK as a forage crop and extends as far north as Ayrshire in south west Scotland.

The yield of miscanthus grown on the Cambridgeshire fens in its 4th year of establishment is reported to have reached 20 t dry weight/ha with a maximum plant height of 2.5 m (Bullard *et al.*, 1997), whilst total biomass, reported for miscanthus in its second year of establishment at a site in Essex, was 25 t dry weight/ha (Beale and Long, 1995). These yields demonstrate the ability of miscanthus to generate large quantities of biomass compared to other potential bioenergy crops such as willow and poplar which have yields of 12.5 and 6.6 t dry weight/ha/year (Armstrong and Johns, 1997). Miscanthus yield potential also compares favourably to the high biomass crop *Berkhya coddii* which can achieve a yield of 22 t/ha/year (Robinson *et al.*, 1997).

Wilkins and Abrutat (1995) reported miscanthus tissue metal concentrations of 16–37 μg Zn/g and 10–17 μg Cu/g when grown on soils contaminated with Zn (292 μg/g) and Cu (188 μg/g). They also reported significant improvement in yield (~ 10 times) when sewage sludge was added to the polluted soil although a similar response was not seen on sewage sludge amended mine waste.

1.3.3 Nettle

Nettle fibre has been reported to be stronger than that of flax and less coarse than that of hemp. A deep rich loam under shaded growth conditions is required to give optimum fibre length and plant yield for nettle, but despite these particular growth condition requirements, nettle was used as a cotton substitute in the manufacture of German army uniforms in 1918 (Grieve, 1931). Nettles are currently being grown as a trial crop for the production of fibres for the textiles industry (Ruckenbauer *et al.*, 2002) and has also been considereed as a source of fibre for the manufacture of automotive interiors (Ellison and McNaught, 2000). Nettles have been shown to accumulate metals from Danube soils in above ground biomass in the decreasing order: Pb>Cr>Cd>Hg (Uhercikova and Hajduk, 1998), Otte and Wijte (1993) reported Cd, Cu and Zn uptakes in nettle of up to 8.5 μg/g, 39 μg/g and 490 μg/g, respectively, in plants grown on the flood plain of the Rhine estuary

1.3.4 Oilseed rape

Oilseed rape is the main oilseed crop grown in northern Europe. Oils produced from agricultural crops are used for lubricants surface coatings and polymers (IENICA, 2000). Some 2.45 million tonnes of oil derived from agricultural sources, mainly oilseed rape and sunflower, is used for industrial applications in Europe annually (Oliver, 2001). Oilseed rape has been cited as a potential crop for transfer of hyperaccumulating traits from *Thlaspi caerulescens* (Brown *et al.*, 1995b).

1.4 Aims and objectives

The objective of this work was to elucidate the potential of flax, miscanthus, nettle and oilseed rape for phytoremediation of land contaminated with Cd, Cr, Cu, Pb, Ni and Zn.

The aims of the work presented in this thesis were:

- to observe the growth response and quantify above ground tissue metal concentration, of flax, miscanthus, nettle and oilseed rape in highly and marginally contaminated soils.

- to indicate the minimum threshold solution concentration which results in the death of flax plants grown in hydroponic culture.

- to assess the influence of genetic variation on growth response to and metal uptake of Cd, Cr, Cu, Pb, Ni and Zn.

- to investigate the response of flax to the addition of chemical agents known to influence plant metal uptake in other plant systems.

2 Materials and methods

2.1 Water

2.1.1 Deionised water

Unless otherwise stated the water was deionised water. The deionised water was prepared by purifying tap water using a mixed anion and cation ion exchange resin, which was housed in a 100 mm × 500 mm column. The deionised water filled a 60-l reservoir tank. The pipe between the column and the tank was fitted with a conductivity meter to monitor the quality of the water. The lowest acceptable water quality was 0.5 M Ω.

2.1.2 Tap water

Untreated tap water was used to water the pot experiment plants and for first rinses during glassware cleaning.

2.1.3 Purite water

Solutions prepared for AA analysis were made up using water deionised by a Purite system (Purite Select Analyst). The heavy metal content of this water was 'guaranteed'<0.0001 mg/l. This will be referred to as Purite water hereafter.

2.2 Glassware

All volumetric glassware used in the study was Analytical Grade B glassware.

Cleaning glassware

All glassware, plastic items and other pieces of equipment which came into contact with solutions or samples were cleaned thoroughly prior to use. Items were soaked for at least twelve hours in 10% Decon 90 solution made up in deionised water. Items were rinsed three times under running tap water and a further three times using deionised water. Items were then dried either in a pie oven (40°C) or, in the case of larger items, on a drying rack. Clean items were stored in closed drawers or cupboards covered in paper towel to prevent contamination with dust or other extraneous material.

Glassware used in acid digestion for atomic absorption (AA) analysis was soaked overnight in Decon 90 solution made up in deionised water, then rinsed three times using tap water. The glassware was then transferred to a 2% nitric acid solution where it was left

to soak for at least 4 hours, before rinsing three times using tap water followed by a further three rinses using deionised water. The glassware was given a final rinse with Purite water. The glass extraction unit for the digestion block was dismantled and similarly washed if it had been used for digests other than nitric acid or aqua regia. Care was taken when the digestion block had been used for metal catalysed digestions, particularly Kjeldahl digests, which were catalysed using copper sulphate and selenium tablets and thus posed a high risk of contamination.

2.3 Matrix components

Plants were germinated and grown in both solution and soil matrices. Solutions were made up in either purite water (germination study; Section 2.5) or nutrient solution (hydroponic studies; Section 2.8–2.11).

2.3.1 Knops nutrient solution salts

Knops nutrient solution, as described by McGregor (1999), was made up using the salts detailed in Table 2.1 in deionised water.

Table 2.1 Salts used in full strength Knops nutrient solution. Weights are the weight of salts added to 22 l of deionised water to obtain full strength nutrient solution. Full strength nutrient solution included 2.2 ml of K solution (Table 2.2).

Salt	*Formula*	*Weight (g)*
Calcium nitrate	$Ca(NO_3)_2 \cdot 4H_2O$	18.04
Iron citrate	$C_6H_6O_7Fe \cdot 3H_2O$	0.52
Magnesium sulphate	$MgSO_4$	10.80
Potassium nitrate	KNO_3	11.00
Potassium orthophosphate	K_2HPO_4	3.80

Knops solution included an addition of a trace element solution (K Solution, Table 2.2).

Table 2.2 Knops trace element solution (K solution). Weights are the weight of salts added to 100 ml of Purite water.

Salt	*Formula*	*Weight (g)*
Boric acid	H_3BO	2.86
Manganese sulphate	$MnSO_4 \cdot 4H_2O$	1.38
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	0.22
Copper sulphate	$CuSO_4 \cdot 5H_2O$	0.08
Molybdic acid	H_2MoO_4	0.09

2.3.2 Component salts of metal-containing solutions

All metal-containing solutions were made up using metal–nitrate salts (Table 2.3).

Table 2.3 Component salts of metal-containing solutions. The purity is the certified percentage purity of the supplied salts. All salts were supplied by Avocado.

Salt	*Formula*	*Purity (%)*
Cadmium nitrate tetrahydrate	$Cd(NO_3)2{\cdot}4H_2O$	99
Chromium (III) nitrate nonahydrate	$Cr(NO_3)3{\cdot}9H_2O$	99
Copper (II) nitrate trihydrate	$Cu(NO_3)2{\cdot}3H_2O$	98
Lead (II) nitrate	$Pb(NO_3)2$	99
Nickel (III) nitrate hexahydrate	$Ni(NO_3)3{\cdot}6H_2O$	99
Zinc (II) nitrate hexahydrate	$Zn(NO_3)_2{\cdot}6H_2O$	99

These salts are deliquescent and/or hygroscopic; therefore, after opening the bottles, Parafilm 'M' laboratory film was used to seal their lids. They were also placed in a desiccator containing silica crystals which was vacuum sealed. Care was taken to weigh the salts out quickly allowing minimal exposure of the salt to ambient air. The nitrate salts were used as they have greater solubility relative to other metal salts.

2.3.3 Salt solution preparation

All salt solutions in the study were prepared as follows unless otherwise stated.

Calculations

The weight of salt required equalled the weight of metal ion multiplied by the weight ratio of salt:metal ion (including any water of hydration). The weight of metal ion required equalled the target concentration multiplied by the final solution volume.

Example: To prepare a 500 mg/l cadmium solution in a 500 ml volumetric flask using cadmium nitrate tetrahydrate. The formula masses of cadmium and cadmium nitrate tetrahydrate are 112.41 and 308.48, respectively.

$$\text{Weight of cadmium (mg)} = 500\ (\text{mg/l}) \times 0.5\ (\text{l}) = 250\ (\text{mg})$$

$$\text{Weight of salt} = \text{weight of cadmium} \times \frac{\text{formula mass salt}}{\text{formula mass cadmium}}$$

$$\text{Weight of salt (mg)} = 250 \times \left(\frac{308.48}{112.41}\right) = 686.06\ (\text{mg})$$

$$\text{Weight of salt (g)} = \frac{686.06}{1000} = 0.6860\ (\text{g})$$

Preparation method

The calculated weight of metal salt was weighed out using a four figure balance. If the weight was greater than 0.5 g, the salt was weighed directly into a beaker. If the weight was less then 0.5 g, the salt was weighed onto an aluminium foil weighing boat then transferred to the beaker with washings. Once the correct weight of salt was in the beaker the solvent matrix was added and the mixture was stirred until the salt had completely dissolved. The stirring was either done manually using a glass rod or automatically using a magnetic stirrer and stirrer bar.

The resulting solution was then transferred to a volumetric flask. The beaker and stirrer bar/rod were then rinsed with the solvent matrix and the rinses transferred to the volumetric flask. The 'washing' procedure was repeated three times. The solvent matrix was then carefully added to the volumetric flask until the bottom of the meniscus was exactly on the mark. The solution was then homogenised by completely inverting the volumetric flask at least nine times.

Additional steps in Knops solution preparation

Knops solution was made up in a 22-l flask. Each of the salts detailed in Table 2.1 were brought into solution separately before addition to the 22-l flask, to avoid co-precipitation of the salts. A 2.2 ml volume of K solution (Table 2.2) was added to 22-l of Knops nutrient solution. Potassium orthophosphate solution was added last, after full dissolution of the other salts, to minimise the formation of phosphate precipitates. Prepared Knops solution was stored in a 22-l flask wrapped in double sided hydroponic NFT sheeting to prevent algal growth. Modification of the Knops solution included: the omission of potassium orthophospate to produce a phosphate free solution; the adjustment of weights of salt added to produce a reduced strength nutrient solution. Where such modifications were made is indicated in the corresponding sections.

2.3.4 Component salts of metal-containing soils

The component metal salts of the artificial soils are detailed in Table 2.4.

Table 2.4 Component salts of metal-containing soils. Salts used to prepare T1–T4 artificial soils (pot experiments; Section 2.6, 2.7). RDHASH is an abbreviation for Riedle-De Haen Ag Seelze-Hennser.

Salt	*Formula*	*Supplier*
Cadmium chloride hemipentahydrate	$CdCl_2 \cdot 2.5H_2O$	Aldrich
Copper (II) chloride hydrate	$CuCl_2 \cdot H_2O$	Aldrich
Lead nitrate	$Pb(NO_3)$	RDHASH
Nickel (II) chloride hexahydrate	$NiCl_2 \cdot 6H_2O$	Aldrich
Potassium dichromate	$K_2Cr_2O_7$	RDHASH
Zinc chloride	$ZnCl_2$	Aldrich

2.4 Plant species

The plant species considered in the study were *Linum usitatissimum* (flax), *Brassica napus* var. *oleifera* (oilseed rape), *Miscanthus* × *giganteus* (miscanthus grass or elephant grass, referred to from now on as miscanthus) and *Urtica dioica* (stinging nettle, referred to from now on as nettle). Flax and oilseed rape seeds were kindly donated by NIAB, Cambridge and miscanthus rhizomes were kindly donated by ADAS, Arthur Rickwood. Nettle rhizomes and seeds were collected from wild populations growing in Glasgow University grounds (Garscube estate). Where appropriate, the agricultural variety names of the species are discussed in the relevant sections.

2.5 Germination of flax and oilseed rape

Two plant species were used in the germination study: flax (varieties Belinka and Viking) and oilseed rape (varieties Martina and Rocket).

2.5.1 Germination procedure

Each of the plant varieties were germinated in Petri dishes (sterile crystal polystyrene single vent 90 mm diameter; Bibby Sterilin) containing vermiculite. Vermiculite was used as it was available in large amounts at low cost relative to the expense of germination paper and it had the water holding capacity necessary to provide a reservoir for the seedlings over the 12-d germination period. To prepare the vermiculite, ~500 ml of vermiculite was ground in a Waring blender for 30 seconds after which it was passed through a 2 mm stainless steel sieve. Four teaspoons (approximately 20 ml) of this fine vermiculite was put into Petri dishes. This provided a moist evenly surfaced mat for germination. Twenty seeds of each variety were sown per Petri dish. Each treatment was replicated five times giving a maximum possible germination of 100 seeds.

2.5.1.1 Micro-growth chambers preparation

Petri dishes were modified to form micro-growth chambers after an initial 3–4-d germination period. Micro-growth chambers were prepared by cutting A4 overhead projector acetates into 15 cm × 28.8 cm pieces (the circumference of the Petri dish being 28.26 cm). The pieces were then stapled into a closed tube, which fitted against the inside wall of the Petri dish exactly. Finally, the lid was placed on top of the tube to provide a chamber with minimal loss of moisture due to evaporation.

2.5.2 Growth phase

Each Petri dish received 20 ml of either a control or metal solution. Purite was used as the control solution. Metals were added as their nitrate salts (Section 2.3.2) and all solutions were made up in Purite water. The metal solution concentrations used in the germination study are detailed in Table 2.5. All the varieties were germinated at the lower of the two concentrations. Additionally, flax, variety Viking, was also treated with the higher metal concentrations (Table 2.5).

Table 2.5 Metal concentrations of the solutions added to the germination Petri dishes. Values shown are solution concentrations added to: (a) each variety of flax (Martina, Rocket) and oilseed rape (Belinka, Viking) and (b) flax (Viking) only.

	Concentration (μg/ml)	
	(a)	(b)
Cadmium	500	1037
Chromium	1000	1560
Copper	500	950
Lead	500	1130
Nickel	500	1000
Zinc	1000	2000

After sowing the seeds and adding solution to the Petri dishes, the lids were replaced to prevent evaporation of the solutions. The Petri dishes were then placed in a growth chamber with a 16-h photoperiod. When the first seedlings germinated, the acetate tubes were placed in the Petri dishes creating the micro-growth chambers. The seedlings were allowed to germinate and grow for a 12-d period after which they were counted. The flax variety Viking seedlings were allowed to grow for a total of 17 days after which they were also counted and in addition, their shoot lengths were measured.

2.6 Pot Experiment One

Elucidating plant responses to highly metal contaminated soil matrices

2.6.1 Preparation of highly metal contaminated soils

The soils used for Pot Experiment One were artificial soils which had been prepared three years earlier by McGregor (1999). These soils were made up to represent a highly metal contaminated spoil type matrix with a high gravel and stone content. The soils were prepared as described by McGregor (PhD Thesis, 1999; Chapter 2). The method is reproduced in Section 2.6.1.1 (shown in Arial font). The control soil was the topsoil/grit mixture described in Section 2.6.1.1 without the addition of the metal salts.

2.6.1.1 Soil preparation (McGregor, 1999)

Five tons of uncontaminated top soil obtained from building excavations was mixed with commercially purchased grit to prepare a topsoil/grit mixture suitable for the experiment. The mixture consisted of approximately ten wheelbarrow loads of soil mixed with five 25 kg bags of medium to coarse grit. After mixing, the mixture was passed through a rotating drum garden shredder to break down the soil aggregates and further mix the material.

The weight of metal salt required to create the chosen soil concentration was calculated on the basis of each pot containing 8 kg of the prepared soil/grit mix. Due to the overall volume of soil used and the relatively dry nature of the soil, no attempt was made to adjust the weight of added salt to take account of the moisture content of the soil.

Metal salts were chosen on the basis of their solubility in water although the availability and cost was also taken into consideration. It was also important that a similar type of salt was used where possible such as metal chlorides. The commercial name and manufacturer of each salt are given in [Section 2.3.2]. The weight of salts added to each pot are detailed in [Table 2.6].

Table 2.6 Weight of salts added to pot soils.

Soil metal treatments (mg/kg)	Formula weight of salt	Weight of salt added to 8 kg of
500 zinc	136.28	8.32
3000 zinc	136.28	49.92
500 copper	170.48	8.44
2000 copper	170.48	33.76
500 nickel	237.71	16.2
1000 nickel	237.71	32.4
300 cadmium	228.34	4.87
1000 cadmium	228.34	16.24
2000 chromium	294.19	45.12
2000 lead	331.20	25.6

Eight kilograms of the prepared planting soil was weighed into a large plastic bucket and a pre-weighed weight of the desired metal salt was emptied into the bucket and mixed thoroughly with the soil. Finally the glass jar containing the metal salt was rinsed with deionised water and the washings were added to the soil. All mixing buckets were washed between different metal treatments.

In total, McGregor prepared 40 × 8 kg pots for each of the zinc, copper, nickel and cadmium soil treatments and 20 ×8 kg pots for the chromium and lead treatments. In the three years prior to Pot Experiment One the soils were used by McGregor to grow trees in a polytunnel in Garscube Estate and received add lib watering during that time.

The pH and loss of ignition of the Pot Experiment One parent soils were established upon preparation. The soil, at a water to soil ratio of 1:2.5, had a pH of 5.3 and, at a 0.01 M $CaCl_2$ to soil ratio of 1:2.5, had a pH of 4.9. The loss on ignition was 7.4% (McGregor 1999).

2.6.1.2 Soil homogenisation

The containers used in Pot Experiment One were window box style pots with internal dimensions of 640 mm x 250 mm x 240 mm. These pots held 28 kg of soil and each treatment was replicated four times, therefore, 112 kg of each soil was required in total. To ensure that the soils used in Pot Experiment One were homogenous, each of the soil treatments prepared and used by McGregor (1999) were re-mixed. Approximately 30 kg of each soil was transferred into a large plastic drum. The drum was then rolled back and forth and tipped end over end to thoroughly mix the soil material. Samples of the homogenised material were placed in plastic bags ready for acid digestion. The remaining soil was transferred to the window box style pots. The plastic drum was rinsed thoroughly three times with tap water before mixing each soil treatment.

2.6.1.3 Final soil metal concentrations

The metal concentrations of the re-mixed Pot Experiment One soils as determined by aqua regia digestion (Section 2.12.1) and AA analysis (Section 2.13) are shown in Table 2.7.

Table 2.7 Pot Experiment One aqua regia digestible soil metal concentrations. Standard deviations are shown (*st. dev.*) with n = 4.

Soil metal treatment	Abbreviation	Total metal concentration (mg/kg air dried soil)	*st. dev.*
Low Cd	Cd_{T1}	326	*46*
High Cd	Cd_{T2}	1,067	*372*
Cr	Cr_{T1}	2,757	*441*
Low Cu	Cu_{T1}	545	*86*
High Cu	Cu_{T2}	2,250	*71*
Pb	Pb_{T1}	2,937	*59*
Low Ni	Ni_{T1}	729	*38*
High Ni	Ni_{T2}	1,348	*137*
Low Zn	Zn_{T1}	731	*63*
High Zn	Zn_{T2}	2,940	*234*

2.6.1.4 Sewage sludge soil

The sewage sludge soil was taken from a sewage farm (Stoke Bardolph). Sewage sludge had been applied to this site for over 50 years resulting in a high metal content. (Rundel and Holt, 1983). This soil was collected form the Stoke Bardolph site and used in the pot experiment study without any further treatment.

2.6.2 Method of growing flax, miscanthus and nettle in highly metal contaminated soils

Pot Experiment One was conducted in a polytunnel within the walled garden at Glasgow University's Garscube estate. The plant species observed were flax, miscanthus and nettle. The flax was sown as seeds whilst the nettle and miscanthus plants were planted as rhizomes. The miscanthus and nettle rhizomes pieces were thoroughly cleaned using tap water prior to planting. Exactly 50 seeds of flax, variety Viking, were sown and four rhizome pieces of both nettle and miscanthus were planted per pot.

To replicate the soil conditions as closely as possible, each of the three plant species was planted in a communal pot which was subdivided into three sections using hardboard cut to fit exactly the internal pot diameter. This ensured that the plant species had identical growth conditions whilst any root interaction was prevented. The plant species were arranged randomly both within pots and within the polytunnel. These random positions were determined by drawing lots. Each pot was replicated four times.

The plants were grown over a 16-week period during which time they were watered such that a moist, rather than dry or waterlogged, soil environment was maintained. The number and maximum height of shoots was recorded throughout the growth period. At the end of the growth period the shoots of all the plants were harvested, however, only the roots from the flax plants were harvested.

2.6.3 Method of growing oilseed rape in highly metal contaminated soils

Latterly, oilseed rape was also sown in pots to ascertain their growth in highly metal contaminated soils. Fifty oilseed rape seeds were sown into 10-inch pots containing the treated soils prepared in the manner described in Section 2.6.2. Each treatment was replicated four times. None of the oilseed rape plants survived in the Pot Experiment One soils.

2.7 Pot Experiment Two

Elucidating plant responses to marginally metal contaminated soil matrices

Pot Experiment Two soils contained the same metals as Pot Experiment One (Cd, Cr, Cu, Pb, Ni and Zn) but lower soil metal concentrations were chosen due to poor growth of the plants in the Pot Experiment One soils. Additionally, dilution of the soil allowed the plant's response to metal concentrations close to the Inter-departmental Committee on the Redevelopment of Contaminated Land (ICRCL) guideline levels for contaminated land (Guidance Note 59/83) to be investigated. The ICRCL threshold levels for the metals in the study are shown in Table 2.8.

Table 2.8 ICRCL 59/83 Trigger concentrations. The group A values shown are for domestic gardens and allotments. The group B values shown are for any land use where plants are grown

	Threshold values (mg/kg air dried soil)
	Group A
Cadmium	3
Chromium	600
Lead	500
	Group B
Copper	130
Nickel	70
Zinc	300

To produce soils at the desired concentrations whilst retaining the aged characteristics of the artificial soils, Pot Experiment Two soils were derived from Pot Experiment One soils. This was achieved by diluting Pot Experiment One soils with a combination of vermiculite and potting sand. This dilution matrix combined the bulking and strong water holding capacity of the vermiculite with the density and free drainage properties of the potting sand. These qualities made the new soil matrices less structurally hostile to the plants than the parent soils.

Four replicates pots were prepared for each plant–treatment combination. Four plant species (flax, miscanthus, nettle and oilseed rape) for each of six metals, each at two concentrations, in addition to three control soils (A, B and C) and a sewage sludge soil resulted in a total of 64 pots.

2.7.1 Preparation of marginally metal contaminated soils

Pot Experiment One soils were passed through a 2 mm steel slab soil sieve and allowed to air dry. Removing the larger stones and water allowed the soils to be accurately diluted.

2.7.1.1. Soil dilution

Each pot contained 400 g of soil matrix: 15 g of vermiculite was added to each pot and the remaining 385 g was made up of Pot Experiment One soil and sand. The weight of sand added was determined by the weight of Pot Experiment One soil required, such that the final combined weight of sand and soil was 385 g (Table 2.9).

Air dried and sieved Pot Experiment One soil (T1), vermiculite and sand fractions were weighed using a two-figure balance and transferred to a plastic bag. The bag was then shaken for 5 seconds to homogenise the matrix before immediately transferring the matrix to the pot. Each of the 16 replicate pots, for each treatment, was prepared individually.

Control soils

Control soils were prepared by dilution of the Pot Experiment One control soil. Two control soils (A and B) were required to reflect the range in dilution factors used in preparing the metal-containing soils. Control soils containing 30% (control soil A) and 10% (control soil B) of the Pot Experiment One soil were, therefore, prepared by dilution with sand and vermiculite (Table 2.9).

A third control soil, collected from the Garscube walled garden (Control soil C), was used undiluted.

Metal-containing soils

The target concentrations for the Pot Experiment Two soils were 100% and 120% of the ICRCL 59/83 (Second Edition) threshold levels for contaminated land. The dilution factor for each soil was calculated by dividing the target concentration (Table 2.8) by the original soil concentration (T1 soils; Table 2.7). The weight of sieved and air dried Pot Experiment One soil (T1) required per pot was the dilution factor multiplied by the total weight of soil per pot (400 g). The weights of T1 soil, vermiculite and sand added to each pot for each treatment are shown in Table 2.9.

Table 2.9 Weights of prepared Pot Experiment One soil, vermiculite and sand added to each experiment two pot. T1 Soil denotes sieved and air dried Pot Experiment One soil. T3 and T4 denote metal soil concentrations 100% and 120% of the ICRCL threshold trigger values, respectively.

	T1 Soil	Vermiculite	Sand
Treatment	Weight added (g)		
Control soil A	120.00	15.00	265.00
Control soil B	40.00	15.00	345.00
Cd_{T3}	3.60	15.00	381.40
Cr_{T3}	87.19	15.00	297.81
Cu_{T3}	95.63	15.00	289.38
Pb_{T3}	68.00	15.00	317.00
Ni_{T3}	38.38	15.00	346.63
Zn_{T3}	164.00	15.00	221.00
Cd_{T4}	4.40	15.00	380.60
Cr_{T4}	104.38	15.00	280.63
Cu_{T4}	114.38	15.00	270.63
Pb_{T4}	81.63	15.00	303.38
Ni_{T4}	46.00	15.00	339.00
Zn_{T4}	196.81	15.00	188.19

Sewage sludge soil

The sewage sludge soil used was described in Section 2.6.1.4.

2.7.2 Method of growing flax, miscanthus, nettle and oilseed rape in marginally metal contaminated soils

Pot Experiment Two was conducted in a greenhouse within the walled garden at Glasgow University's Garscube estate. The plant species observed were flax, miscanthus, nettle and oilseed rape. Flax, nettle and oilseed rape were sown as seeds whilst miscanthus was planted as rhizomes. Fifty flax seeds (variety Viking) were sown into each of four replicate pots for each of the soil treatments. Similarly, 30 nettle seeds and also 20 oilseed rape seeds (variety Synergy), were sown into each of the species' four replicate pots for each soil treatment. For miscanthus, one rhizome piece with several buds was selected and planted into each of the four replicate pots for each soil treatment.

The plants were grown over a 15-week period. After sowing, the pots containing flax, nettle and oilseed rape, were covered with plastic bags to retain moisture and therefore promote germination. The bags were removed on an *ad hoc* basis once several seedlings had emerged from each pot. During week five (Day 40), it was necessary to replant some of the miscanthus plants as the rhizome pieces did not produce any shoots. In week five,

each pot was fertilised using 50 ml of full strength Knops solution. For the remainder of the growth period, each species was grown without further nutrient addition. Water was supplied to maintain a moist soil environment.

During week eight, the oilseed rape plant were subject to severe herbivorous attack by cabbage white caterpillars. The oilseed rape plants were successfully treated using a malithion insecticide according to the manufacturers instructions: additionally the other plant species were treated prophylactically.

At the end of the growth period the numbers of surviving plants in each pot were recorded as were the heights of the flax and miscanthus plants. The shoots of all the plants were harvested.

2.8 Nutrient film technique (NFT) study

2.8.1 NFT System

The system comprised 14 specialist NFT channels (132 × 15 × 8 cm), 14 small pumps (5 watts Mini jet Aquarian Systems), pipe and 56 right angle pipe fittings, all purchased from Sunlighter Systems. Fourteen 5-l reservoir bottles (thoroughly rinsed Decon 90 containers) and a metal frame (Handy angle) completed the system (Fig. 2.1). A hole was cut in the top of the 5-l reservoir containers to allow access of the pump. The NFT channels had ridged floors to allow the solution to run along the grooves in an even, regular flow. A large filter funnel was placed in the lid of the reservoir container to catch the solution flowing from the end of the NFT channel. The system was assembled as shown in Figure 2.1. Throughout the NFT experiment the slope of the channels was maintained at 10%.

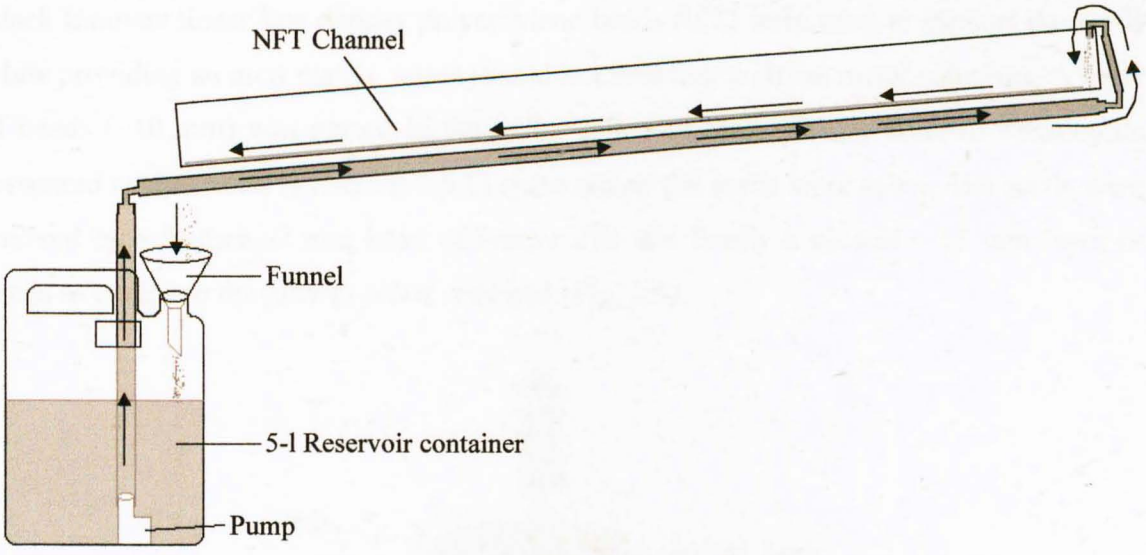

Figure 2.1 NFT system set up. The diagram shows the circulation of NFT solution (■). The direction of flow is indicated by the arrows. The equipment is not drawn to scale.

2.8.2 Hydroponic growth collars

Brown plastic drainpipe with an internal diameter of 70 mm was purchased from a plumber's merchant. The pipe was cut into 30 mm lengths. Ballet netting with a mesh size of 2.5 mm, purchased from a haberdashery, was used as a membrane for the base of the collar to contain the growing matrix and the seeds. Shorter pieces of drainpipe were cut into 20 mm sections, then cut along their lengths to form a split ring. The ballet netting was glued to the outside 30 mm section then the 20 mm spilt ring was glued in place round the outside of the 30 mm section to clamp the ballet netting securely. The growth collar was held tightly with an elastic band whilst the glue set. This produced a cheap effective container for the support matrix and seeds (Fig. 2.2).

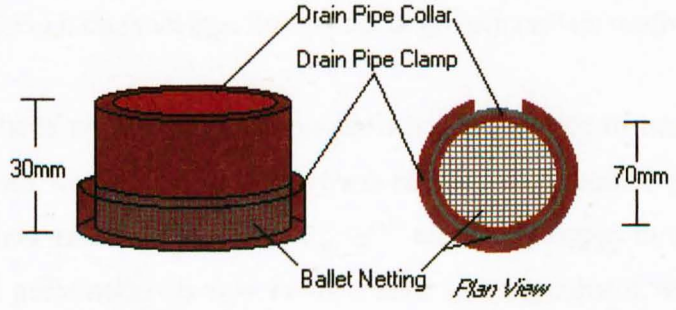

Figure 2.2 Hydroponic growth collars.

Black Innovex linear low density polyethylene beads (ICI) were used to support the seeds while providing an inert matrix which would not interfere with the metal solutions. A layer of beads (~10 mm) was placed in the collar followed by a ~2 mm layer of vermiculite (prepared as described in Section 2.5.1) on to which the seeds were sown. The seeds were covered by a further ~2 mm layer of vermiculite and finally a second ~ 15 mm layer of beads to complete the growth collar seed bed (Fig. 2.3).

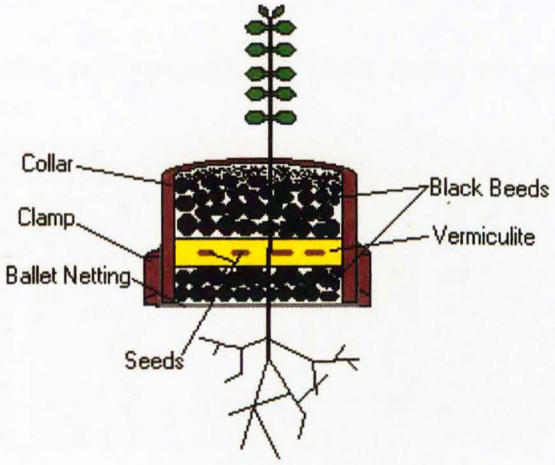

Figure 2.3 Cross section of a hydroponic growth collar seed bed.

The NFT system was covered using specialist plastic NFT sheeting (Sunlighter Systems). The sheeting had a white surface with high albedo, which was placed uppermost to reflect the heat and light energy, and a dark surface placed facing the nutrient solution to minimise algal growth.

2.8.3 NFT Growth phase

There were 14 channels in the NFT system available for the experiment. Two metals at six different concentrations could, therefore, be observed in separate channels simultaneously: the seventh and fourteenth channels contained a control solution for each of the two metals. Each channel had five replicate growth collars uniformly spaced along its length.

Flax plants were established in growth collars prior to transfer to the NFT system. Fifty flax seeds were sown in each growth collar and the collars placed in seed trays. The collars were then moistened by applying 2/5th strength Knopps nutrient to the top of the collars to initiate germination before being placed in an incubator with a 16-h photoperiod at 25°C for 14-d. After the 14-d establishment period, the growth collars were transferred into the NFT system. The NFT system was located in a greenhouse where a 16-h photoperiod was maintained using 400 watt mercury halide growth lights (Philips HP1 Plus Bus B, Sunlighter systems). The temperature was maintained at 20°C throughout using a thermostatically controlled heater.

Five litres of metal solutions, of the concentrations detailed in Table 2.10, were prepared in 2/5th strength phosphate free Knops nutrient. Solutions of each of the six concentrations for two metals were prepared simultaneously. Once prepared these solutions were decanted into the 5-l NFT reservoir containers. The solutions were then run through the NFT system using the pumps. After 7 d the reservoir containers were replaced with containers filled with freshly prepared metal solutions.

Table 2.10 NFT solution concentrations. Values shown are the target concentrations for the NFT solutions.

Metal	Target solution concentration (μg/ml)					
Cd	1	2	5	10	15	20
Cr	0.025	0.1	0.25	0.5	1	2
Cu	1	2	5	10	15	20
Pb	4	10	20	40	60	80
Ni	0.2	0.6	1	2	6	10
Zn	10	20	40	60	75	100

After 14 d growth in the NFT system, the number of surviving plants and the extent of plant chlorosis relative to the control plants were recorded.

2.9 Flax varietal comparison study

Twelve varieties of flax were used in the flax varietal comparison study: Argos, Ariane, Diane, Electra, Elise, Escalina, Evelin, Hermes, Martta, Rasia, Viking and Viola. Each variety was grown in a separate growth collar (Section 2.8.2) into which 50 seeds were sown. The 12 collars were placed in a seed tray containing either the control solution or a metal treatment solution.

The metals used in the flax varietal comparison study were Cd, Cr, Cu, Ni, Pb and Zn; a control solution was also included. The metals solutions were made up from their nitrate salts (Section 2.3.2). The solutions were made up in 2/5^{ths} strength phosphate free Knops solution (Section 2.3.1). The target concentration for each metal is shown in Table 2.11. Each treatment was replicated four times. To ensure identical growing conditions for each of the treatments, a seed tray for each metal and one control tray were grown simultaneously. This procedure was repeated four times. Owing to limited growth space, it was not possible to grow four replicates of each metal treatment concurrently, thus, the replicate treatments were grown consecutively.

Table 2.11 Flax varietal comparison study target solution metal concentrations.

Treatment	*[Phosphate free Knops solution]*	*Target [metal] (µg/ml)*
Control	2/5ths strength	-
Cd	2/5ths strength	5.00
Cr	2/5ths strength	1.00
Cu	2/5ths strength	2.00
Pb	2/5ths strength	40.00
Ni	2/5ths strength	1.00
Zn	2/5ths strength	10.00

Seven seed trays were prepared simultaneously allowing one tray for each treatment and one control tray. Once prepared and sown, the trays were watered using the prepared metal or control solution. Prior to germination, control and metal-containing solutions were added to the top of the collar in order to moisten the vermiculite seedbed layer. The trays were then placed in an incubator at 25°C with a 16-h photoperiod for 7 d. Post-germination, the solutions were added directly into the seed tray rather than watering from above, to avoid contamination of the stem and leaf material with any metal solution. After the 7-d period in the incubator, the plants were moved to a greenhouse where a 16-h photoperiod was maintained using 400 watt mercury halide growth lights (Philips HP1 Plus Bus B, Sunlighter systems). The temperature was maintained at 20°C throughout using a thermostatically controlled heater. For the first two days the seed trays were watered with $2/5^{ths}$ Knops solution containing phosphate (Section 2.3.1), to prevent the plants suffering from phosphate deficiency. After the 2-d growth period in the normal $2/5^{ths}$ strength Knops solution, the solutions were replaced with the metal-containing solutions once more. The plants were then grown in these metal-containing solutions for a further 19-d period, during which time the trays were topped up with the treatment solution according to the plants requirement for water. Depending on the rate of evapotranspiration, the seed trays received between six and eight litres of treatment solution. The total growth period was 28 d during which time the plants were exposed to the metal solutions for 26 d.

After the 4-week growth period, the maximum plant height and fresh weight of stem and leaf material, in each collar, was measured to allow comparisons between varieties to be made. Additionally, signs of chlorosis were recorded. The plants in each collar were harvested and separated into two fractions: a stem and leaf fraction and a root fraction. . The plant material contained within the internal volume of the collar was discarded along with the black beads and vermiculite support material to prevent any vermiculite

contamination in the subsequent acid digests. The plant fractions were then washed under tap water and rinsed with deionised water and any residual vermiculite attached to the roots, carefully removed. The plant material was then put in folded 25 cm Whatman No.1 filter paper envelopes and oven dried (80°C). Once dry, the stem and leaf material was weighed (shoot dry weight) and the root material was also weighed (root dry weight). The dried shoot and root material from each variety was stored in a self seal plastic bag until required.

The oven dried root fractions and stem and leaf fractions, for each of the replicate treatments, were pooled to give a bulk sample which was then prepared for acid digestion and AA analysis (Section 2.12). The varieties grown in the flax varietal comparison study did not yield sufficient root material to allow replication of the acid digestion analysis, however, a single bulk sample from all the replicates was prepared and analysed.

2.10 Buthionine sulfoximine (BSO) study

Collars, as described in Section 2.8.2, were sown with 50 flax seeds of variety Viking. The collars were then placed in an incubator at 25°C with a 16-h photoperiod for 7 d to allow the seeds to germinate before transferral to Kilner jars containing the treatment solutions (Table 2.12). Each treatment was replicated six times.

Table 2.12 Treatment solutions used in the BSO study. Values represent the target concentrations for Cd and BSO. Each treatment solution was made up in phosphate free Knops nutrient solution to a final strength of 2/5ths normal strength (Section 2.3). Buthionine sulfoximine (BSO) was added at either 100 μM or 25 μM and Cd was added at 44.5μM.

Treatment	*[Phosphate free Knops solution]*	*Target [Cd]*	*Target[BSO]*
Control	2/5ths strength	-	-
Cd	2/5ths strength	44.5 μM (5 μg/ml)	-
BSO(a)	2/5ths strength	-	100 μM
BSO(a) + Cd	2/5ths strength	44.5 μM (5 μg/ml)	100 μM
BSO(b) + Cd	2/5ths strength	44.5 μM (5 μg/ml)	25 μM

The Kilner jars (1.5-litre) were wrapped in aluminium foil to prevent any light from stimulating algal growth within the jars. The necks of the Kilner jars had the same internal diameter as the external diameter of the growth collars, thus, the collars were supported when placed directly in the neck of the Kilner jars. Care was taken when transferring the seedlings to the Kilner jars not to damage their delicate root systems.

The Kilner jars were then placed in the greenhouse where they were given a 16-h photoperiod using 400 watt mercury halide growth lights (Philips HP1 Plus Bus B, Sunlighter systems). The temperature was maintained at 20°C throughout using a thermostatically controlled heater.

The plants were grown for a 14-d period during which the nutrient solution was replenished to prevent the plants drying out. However, evaporation from the Kilner jars was minimised by the seal provided by the tight fit of the collars in the neck of the jars. Representative samples of the solution were taken during the growth period for AA analysis (Fig. 6.4; Section 6.1.2). Additionally, the Kilner jars were shaken daily to ensure the treatment solutions remained homogeneous.

After the 14-d growth period, the plants were harvested. For each collar the number of shoots and the fresh weight of shoot tissue were recorded. The shoots were then dried in a pie oven (40°C). The collars were removed from the Kilner jars, again taking care not to break the brittle hydroponic roots. Roots were collected from the base of the collars by being torn off. Cutting the roots from the collars was not a viable option as this sampling method would have destroyed the netting, preventing re-use of the collars and allowing the vermiculite and beads to spill out and contaminate the root sample. The roots were washed carefully to prevent loss of the root material but thoroughly to remove all traces of vermiculite and the nutrient solution. Washing of the roots included three rinses with tap water, followed by two rinses with deionised water and finally a rinse with Purite water. For each treatment, the roots from each of the five collar replicates were pooled as the yield was too low for separate analysis. The roots were then placed on clock glasses and dried in a pie oven (40°C). The dry shoot and root material was weighed and stored in self seal plastic bags.

2.11 Histidine study

The experimental procedure for the histidine study was conducted in the same way as for the B.S.O. study (Section 2.10). Two criteria were modified: the treatment solutions (Table 2.13) and the length of the growth period which was extended from 14-d to 19-d.

Table 2.13 Treatment solutions used in the histidine study. Values shown are the mM target concentrations for Ni, Cu and histidine (His) in Each treatment solution was made up in phosphate free Knops nutrient solution to 2/5ths normal strength (Section 2.3). The metal concentrations were equivalent to 9.98 μg/ml and 21.60 μg/ml for Ni and Cu, respectively. The solutions were made up in 1.5 l Kilner Jars.

Treatment	*Phosphate free Knops solution*	*[Ni]*	*[Cu]*	*[His]*
Control	2/5ths strength	-	-	-
Histidine	2/5ths strength	-	-	0.34 mM
Nickel	2/5ths strength	0.17 mM	-	-
Copper	2/5ths strength	-	0.34 mM	-
Histidine + Nickel	2/5ths strength	0.17 mM	-	0.17 mM
Histidine + Copper	2/5ths strength	-	0.34 mM	0.34 mM

2.12 Sample metal analysis

Plant and soil analyses were conducted according to the Glasgow University A.F.E. Chemistry Department standard protocol. The analysis protocol included sample preparation, sample digestion and atomic absorption analysis. Extraction procedures were also used.

2.12.1 Sample preparation

2.12.1.1 Soil preparation

Soils and spoils were air dried on clean plastic sheeting before sieving through a 2 mm steel sieve. A ~ 50g representative sub-sample of sieved soil was ground in a mortar and pestle to a fine powder before being stored in a self seal plastic bag.

2.12.1.2 Plant preparation

Plant samples were washed three times with tap water, followed by two rinses with deionised waste and given a final rinse with Purite water. The plant sample was dried in a pie oven (40°C, 12 h) to remove the bulk of the moisture before transferral to an oven set at 80°C for 12 h. The sample was then ground in a hammer cutter mill (Glen Creston "Cullattic") with a mesh size of 1 mm. Bulky or woody samples were ground in a larger hammer cutter mill (Fritsch 'Pulverisette 19') which had a mesh size of 2 mm. Separation of fibrous components of plant tissues occurred during milling of some samples (particularly flax). Manual cutting of this fibrous material into short lengths using scissors was necessary before mixing the material back into the milled sample. The homogenised sample was then stored in a self seal plastic until required.

2.12. 2 Soil extraction

Soils were extracted using solutions of increasing strength to give an indication of the plant availability of the soil metals. These extracting solutions, in order of increasing strength were: 0.5 M calcium chloride ($CaCl_2$), 0.05 M ammonium EDTA (EDTA) and aqua regia. The $CaCl_2$ and EDTA extractions were carried out sequentially whereas the aqua regia digest was conducted using a fresh soil sample.

2.12.2.1 Calcium chloride and EDTA preparation

Salt solutions were prepared as described in Section 2.3.3. A two figure balance was deemed sufficiently accurate for the weighing of the salts. The solvent matrix was 'Purite' water. Analar ammonia solution (BDH) was added to the NH_4EDTA solution to promote the dissolution of the EDTA salt, prior to adjusting the salt solution to pH 7.

2.12.2.2 Extraction procedure

A sub-sample of ~2 g of prepared soil material was weighed accurately using a four figure balance and transferred to a 4-oz glass jar. A 20-ml volume of either the $CaCl_2$- or EDTA-extracting solution was added to each jar using a calibrated dispenser. The contents of the 4-oz jar was then shaken for 16 h on an end over end shaker. At the end of the 16-h shaking period, the extracts were filtered through Whatman No. 1 filter papers into plastic bottles and stored until required. The procedure was carried out for each soil sample in triplicate.

2.12.3 Acid digestion

2.12.3.1 Aqua regia digestion of soil material

Acid digestion of soil was possible using an aqua regia solution consisting of a 3:1 ratio of hydrochloric acid (HCl):nitric acid (HNO_3). The acids used were 6 M HCl ('AnalaR', 35.4%, BDH) and 69% HNO_3 ('AnalaR', 68.5–69.5%, BDH).

Approximately 0.25g of the soil sample was weighed out on an aluminium weighing boat using a four figure balance; the exact weight was recorded to four decimal places. The sample was transferred carefully into an acid digest tube. The tube was then tapped once on the bench to ensure all sample rested at the base of the tube. The aluminium weighing boat was then re-weighed to allow the precise weight of sample, to four figures of accuracy, to be recorded. After the second weight was recorded, the aluminium weighing boat was cleaned using a balance brush before continuing with the next sample. The procedure was carried out for each soil sample in triplicate.

Aqua regia (10 ml) was dispensed into each acid digest tube and then left, covered with paper towel, for at least 12 h, in a fume cupboard to allow the acid to equilibrate with the soil. The tubes were then transferred to a digest block (Tecator Digestion System 40 1016 Digester). A total of 40 tubes could be accommodated per run, out of which three blanks, at least, were included. The digest block was set to and thereafter thermostatically

maintained at 125°C. An extraction unit designed to fit in the necks of the digestion tubes was used to remove NO_2 gas evolved during the digestion process. The digestion block was run for at least three hours or until gas emissions ceased, whichever took longer.

Once the digestion was complete, the block was switched off and the tubes allowed to cool. The contents of the tubes, with Purite washings, were then filtered through hardened Whatman No. 50 filter paper into 25-ml or 50-ml volumetric flasks. The smaller 25-ml flasks were used when the concentration of the metal in the soil was anticipated to be low. The filter papers were allowed to drain fully then these were also rinsed with Purite water to ensure all the solution was transferred into the volumetric flask. The volumetric flasks were then allowed to cool before making up to the mark.

2.12.3.2 Nitric acid digestion of plant material

Plant digests were conducted in the same way as soil digests (Section 2.12.3.1) with the following exceptions:

The dried ground plant material was digested using 69% nitric acid rather than aqua regia. The acid used was BDH 'AnalaR' nitric acid. The digest block was set to a lower temperature of 120°C. All plant digests were filtered into 25-ml volumetric flasks.

2.12.4 Flame atomic absorption analysis

The atomic absorption spectrophotometer (AAS) used for all the analyses in the study was a Perkin Elmer 1100B spectrophotometer. The spectrophotometer was used in normal Acetylene/Air Flame mode.

2.12.4.1 AA standards

The atomic absorption spectrometer was calibrated using a range of standard solutions for each element in the study. Atomic absorption standard solutions were made up in 100-ml volumetric flasks. With the exception of lead this required an intermediate dilution step to bring the solutions into the necessary range. Two sets of standard solutions were made up for every element in the study. One of these sets were calibration standards the other set were a set of check standards. The standard solutions used were BDH 'Spectrosol' solutions and Reagecon 'AAS Standard Solutions'. Both of these manufacturers produce standard solutions as a 1000 ppm solution of the metal nitrate salts in 0.5 M nitric acid. Each metal standard was made up separately. All AA standards were made up in the matrix of the solutions to be analysed.

The Perkin Elmer 1100B spectrophotometer theoretically allows calibration up to six times the linear range, however in practice the machine did not give a sufficiently stable response for all the elements over this range. Each element was therefore calibrated using the following standards.

Cadmium and Nickel

Cadmium and nickel had a linear range of 2 ppm and the response of the calibration standards was sufficiently stable and accurate response to allow calibration up to three times the linear range. Cadmium and nickel standard solutions were made up at 2 ppm and 6 ppm; check solutions were made up at 1 ppm and 4 ppm. The limit of detection of 0.02 ppm.

Chromium and Copper

Copper and chromium had a linear range of 5 ppm and the response of the calibration standards was only sufficiently stable and accurate to allow calibration up to the linear range. Chromium and copper standards were made up at 5 ppm; check standards were made up at 3 ppm. The limit of detection of 0.05 ppm.

Lead

Lead had a linear range of 20 ppm making it the least sensitive of all the elements in the study. The response of the calibration graph was only sufficiently stable and accurate up to the linear range. The calibration standards were made up to 20 ppm and the check standards were made up to 10 ppm The limit of detection for lead was 0.2 ppm.

Zinc

Zinc was the most stable element in the study and was the only element which gave a sufficiently stable response to allow calibration to six times the linear range. Zinc also had the lowest linear range of all the elements in the study of 1 ppm. It was possible to use calibration standards up to 6 ppm the check solutions up to this range being accurate to within 5%. Zinc calibration standards were made up at 1 ppm, 3 ppm and 6 ppm; check standards were made up at 0.5 ppm, 2 ppm and 5 ppm. The limit of detection for zinc was 0.01 ppm.

Table 2.14 Limit of detection of elements in solution by AA. The corresponding limits of quantification in tissue and soil samples are also shown. These figures are based on a 0.2500 g sample digested and made up to a volume 25.00 ml.

Element	Atomic adsorption solution (mg/l)	Minimum detectable tissue/soil concentration (μg/g)
Cd	0.02	2.00
Cr	0.05	5.00
Cu	0.05	5.00
Pb	0.20	20.00
Ni	0.02	2.00
Zn	0.01	1.00

2.12.4.2 AA Calibration

The calibration of the machine was checked at the start of each run and then re-checked every 20 samples. The AA was auto zeroed initially after each sample was run and if the machine was drifting either up or down then the auto zero was used after each sample, however, it was usually only necessary to auto zero the machine after every third sample.

2.12.4.3 AA Sample analysis

The burner head position was always optimised at the start of each batch of analysis although it was not necessary to do this between elements. If the sensitivity check fell below the minimum acceptable value then the burner head was cleaned using a sonic bath (Sonicor 50/60Hz) and the nebuliser was optimised, before restarting the machine.

Care was taken to ensure the samples, and any dilutions, were homogenised before analysis by inverting each flask nine times. The solutions were not shaken as air bubbles suspended in the matrix caused an underestimate of the true concentration. The AA was set to read each sample three time then give an average result. The aspiration time was three seconds. The machine was auto zeroed at regular intervals or at least every third sample. The top standard was checked, to ensure the top of the calibration graph was not drifting, every 20 samples or at least after 40 samples if the zero was stable.

3 Plant growth initiation

The initiation of plant growth was investigated using flax, miscathus, nettle and oilseed rape. However, a detailed investigation of germinations was conducted using only species that had commercially available seed: flax and oilseed rape. The other two species, miscanthus and nettle, were rhizomatous species and thus were propagated using rhizome pieces. This chapter will focus primarily on the ability of flax and oilseed rape seeds to germinate in the presence of heavy metals.

3.1 Germination of flax and oilseed rape

3.1.1 Micro-growth chamber development

During the initial method development for the germination procedure, the seeds were placed in a Petri dish containing vermiculite (Section 2.5). However, seed germination was not synchronous. Consequently, the first germinating seedlings raised the Petri dish lids which lead to rapid evaporation of the solutions. This evaporation was a source of non-uniform variability which it was necessary to eliminate from the experiment.

The Petri dish method not only provided a cheap and compact germination compartment but also allowed ease of replication and was convenient for the addition of the metal solutions. For these reasons and due to the limited space in the growth cabinet, a propagator was not considered a practical alternative. Thus, the Petri dishes were modified using overhead projector acetates to create 'micro-growth chambers' (Section 2.5.1.1). These chambers allowed for 150 mm of growth height by the seedlings whilst maintaining a sealed growth chamber, thereby preventing loss of moisture. The transparency of the chambers allowed the seedlings full exposure to the 16-h photoperiod.

3.1.2 Germination study

The germination study was designed to establish whether or not the seeds of flax and oilseed rape could germinate in the presence of Cd, Cr, Cu, Pb, Ni or Zn in solution. As an initial investigation into the feasibility of flax and oilseed rape to act as phytoremediating crops, the germination rate of two varieties of each species in the presence of the six metals was observed. The flax varieties used were Belinka and Viking. The oilseed rape varieties used were Martina and Rocket. The seedlings were germinated over a period of twelve days.

In addition, the flax variety Viking was considered in more detail than the other varieties: both its germination and shoot length responses to solution concentrations of two strengths were observed after 17 days. Positive germinations for all other varieties were recorded when the root and shoot lengths were both > 1 mm. The metal concentration of the solutions are detailed in Section 2.5.2 (Table 2.5).

3.1.2.1 Germination in control solutions

The control plant germination of flax variety Viking and oilseed rape varieties Martina and Rocket were consistently high (Fig. 3.1), with all varieties giving a germination ≥ 95% of the total number of seeds. The germination for flax variety Belinka was lower at an average of 86%.

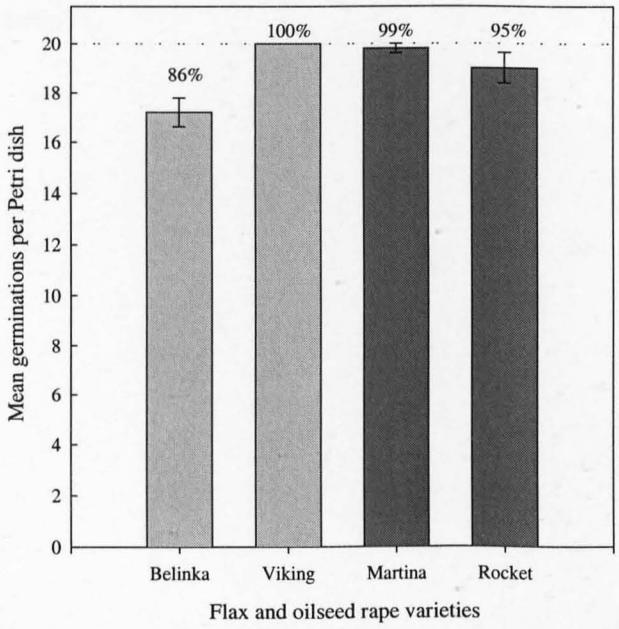

Figure 3.1 Germination of flax and oilseed rape varieties in control solutions. Mean germinations of flax (▪) and oilseed rape (▪) varieties per Petri dish are shown and values above bars represent % germination. Error bars represent SE of mean. Maximum number of possible germinations (20) indicated by ----.

3.1.2.2 Germination in metal solutions

Initial germination study

Comparison of seedling germination in the control solution (Fig. 3.1) with those of the metal solutions (Fig. 3.2) revealed that there was little impact on the germination of all varieties for five out of the six metals studied. The exception was the germination of varieties exposed to the Cr solutions; seeds of all four plant species exposed to the Cr solution (1000 μg/ml) had lower germinations than the seeds exposed to the control solutions (Fig. 3.1, 3.2) this difference was significant (*t* test, $P<0.05$) for Viking, Martina and Rocket. In the Cr solution, the flax variety Viking had the smallest reduction in germination (5%) whilst the oilseed rape variety Martina had the greatest reduction (23%). None of the other metals in the study, when compared to the control, gave significant reductions in germination.

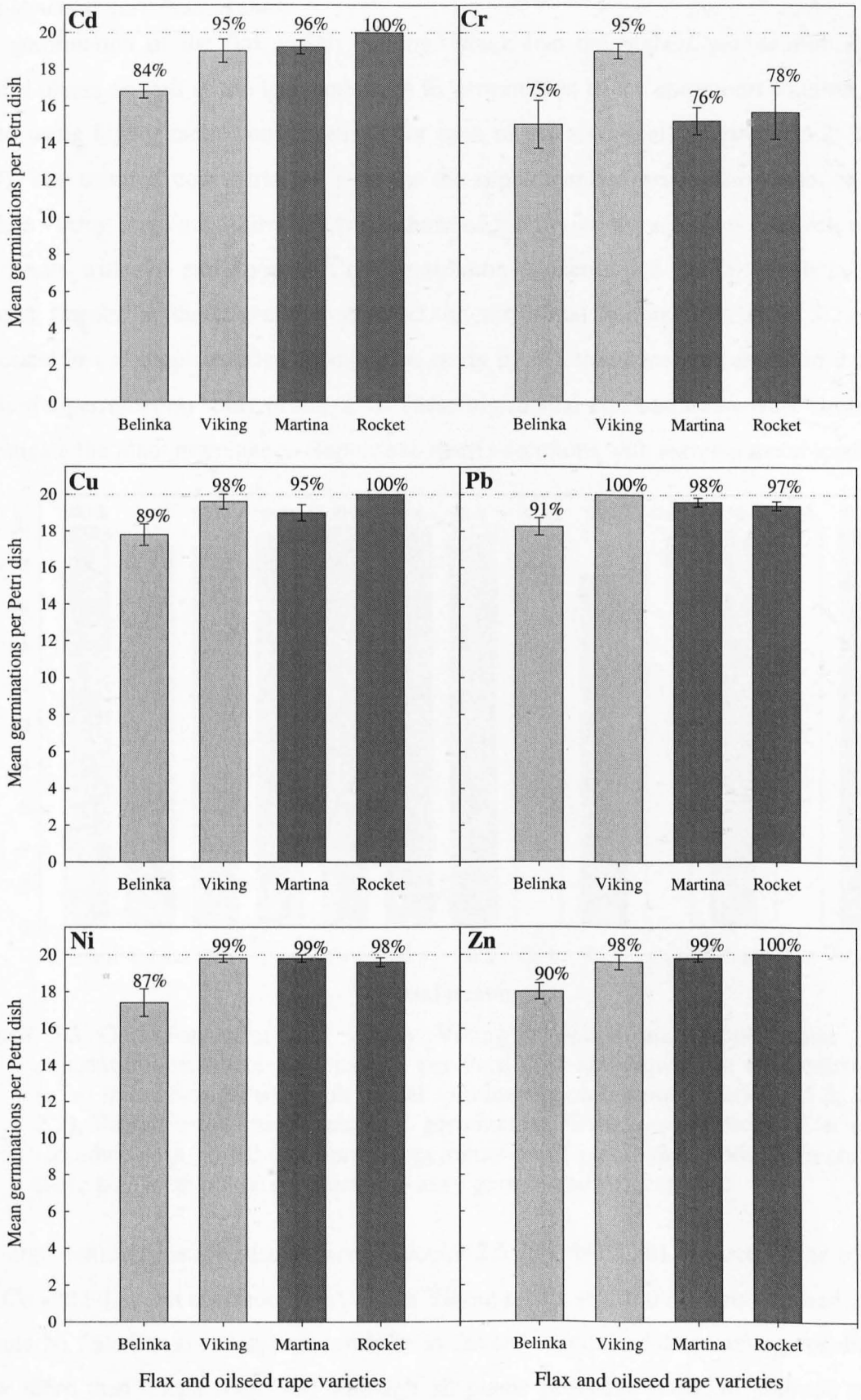

Figure 3.2 Germination of flax and oilseed rape varieties in metal-containing solutions. Mean germinations of flax (▒) and oilseed rape (■) varieties per Petri dish are shown and values above bars represent % germination. Error bars represent SE of mean. The metal concentrations used are detailed in Section 2.5.2; Table 2.5a. Maximum number of possible germinations (20) indicated by ----.

Supplemental germination study

The germination of the flax variety Viking, which had the highest germination in the control plants as well as the least reduction in germination in the chromium solution, was tested using higher metal concentrations for each of the six metals (Section 2.5.2; Table 2.5b). The solution concentrations used for the supplementary germination tests on flax variety Viking were approximately twice those of the initial test solutions for each of the six metals with the exception of Cr. The solution concentration of Cr, which had the greatest impact, at the concentrations used, on the initial germinations (Fig. 3.2), was increased in the supplementary germination study by 1.5 times the concentration used in the initial germination study (Table 2.5). These high metal concentrations were chosen to investigate the plant germination response to matrix solutions with extreme metal loadings.

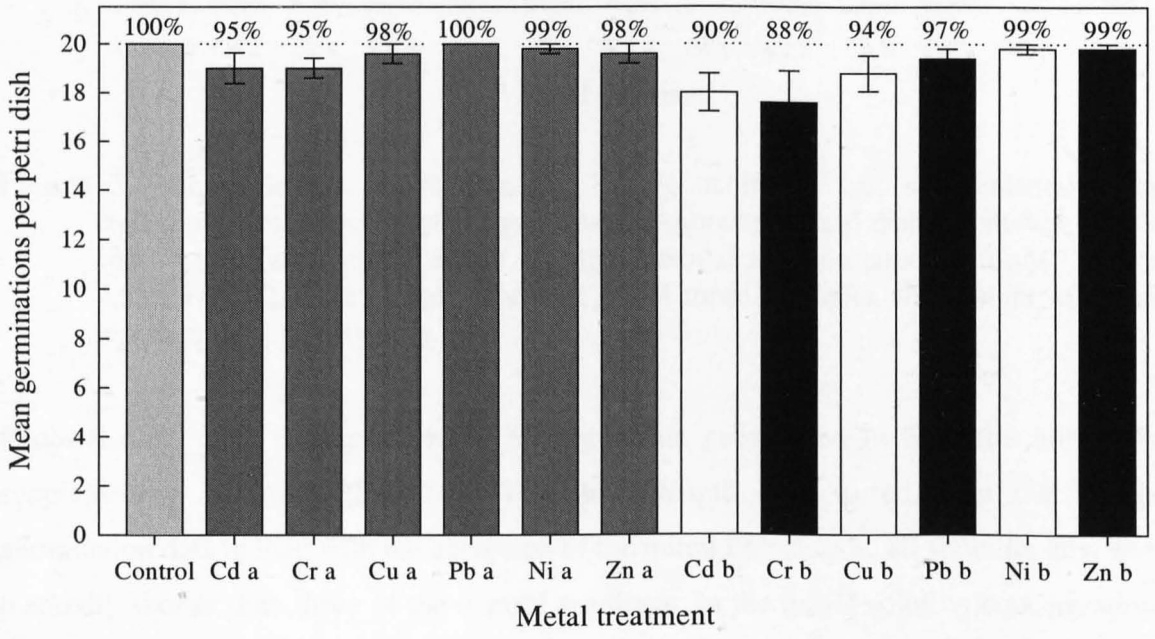

Figure 3.3 Germination of flax variety Viking at initial and supplemental metal concentrations. Mean germinations per Petri dish are shown for: the control (▪), (a, ■) initial and (b, ■) supplemental solution concentrations (Section 2.5.2; Table 2.5). Values above bars represent % germination. Error bars represent SE of mean. Columns with no fill (□) represent germination of plants that produced roots only these plants cannot be considered to have germinated successfully.

The high solution metal concentrations (Section 2.5.2; Table 2.5b) of three of the metals, Cd, Cu and Ni, had a dramatic effect on the Viking plants seeds: the plants exposed to Cd, Cu and Ni failed to germinate successfully as the shoots either did not emerge or did not grow more than 1 mm (Fig. 3.3) although all plants produced roots. In addition to the failure of the plants in the Cd, Cu and Ni solutions to germinate, the Viking plant germination in the Cr solution fell by 12%, the germination rates in both the Cr a and Cr b (Fig. 3.3) were significantly different from the control (*t* test $P<0.05$). The marginal decrease in germination observed for Viking seeds exposed to the high Pb and Zn solutions was not significant.

3.1.2.3 Shoot length of Viking seedlings

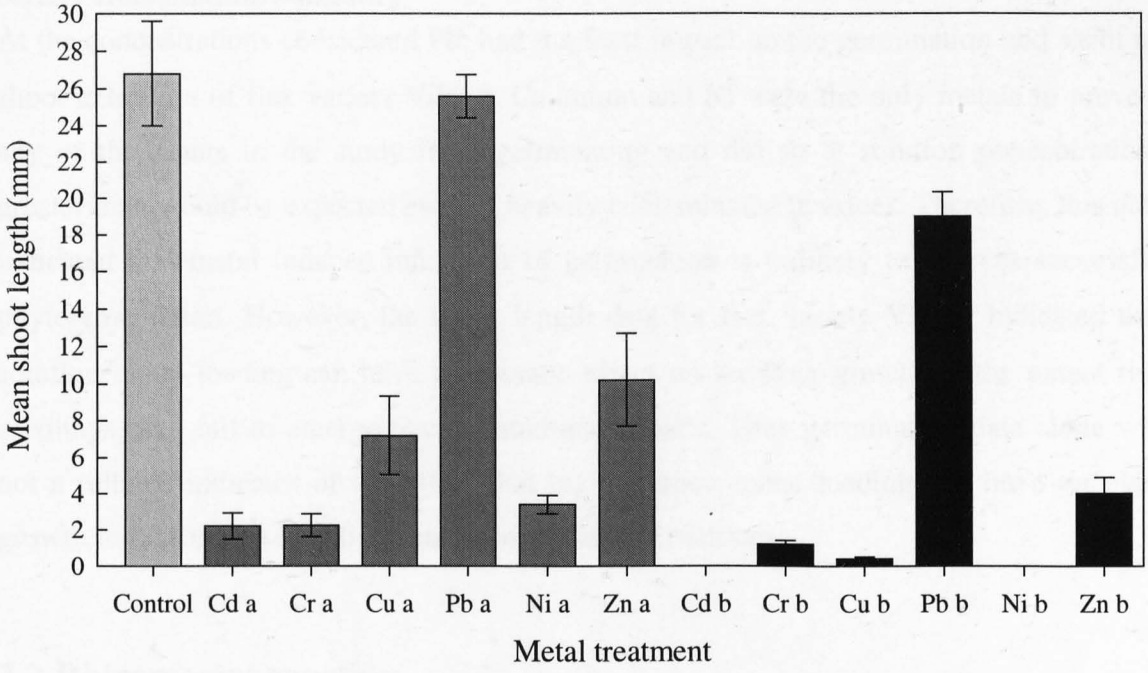

Figure 3.4 Shoot length of flax variety Viking at initial and supplemental metal concentrations. Mean length of germinating shoots per Petri dish are shown for: the control (▪), (a, ▪) initial and (b, ▪) supplemental solution concentrations (Section 2.5.2; Table 2.5). Error bars represent SE of mean. Samples with height <0.5 mm represent root growth only.

Shoot lengths were recorded for the Viking plants germinated in both the initial and supplementary solutions (Fig. 3.4). The shoot length data varied from that of the germination data in that, with the exception of the initial Pb solution, all shoot lengths were markedly shorter than those of the control seedlings. In the initial solution concentrations (Section 2.5.2; Table 2.5a) the shoot lengths decreased in the following order:

Pb ≫ Zn >Cu > Ni > Cd = Cr

Whilst in the supplementary solution concentrations (Section 2.5.2; Table 2.5b) the shoot lengths decreased in the following order:

Pb ≫ Zn > Cr >Cu > Cd = Ni

Lead was the metal which had the least impact on shoot extension in both the initial and more concentrated solutions at the concentrations considered, followed by zinc.

3.1.2.4 Germination summary

At the concentrations considered Pb, had the least impact on the germination and seedling shoot extension of flax variety Viking. Cadmium and Ni were the only metals to prevent any of the plants in the study from germinating and did so at solution concentrations greater than would be expected even in heavily contaminated matrices. Therefore, this data indicated that metal induced inhibition of germination is unlikely to prevent successful phytoremediation. However, the shoot length data for flax variety Viking indicated that solution metal loading can have a dramatic effect on seedling growth to the extent that seedlings may fail to emerge from contaminated soils. Thus germination data alone was not a reliable indicator of the effect that high solution metal loading can have on plant growth initiation and establishment in contaminated matrices.

3.2 Rhizome propagation

Miscanthus and nettle are rhizomatous plants which can be vegetatively propagated using rhizome pieces. It was not possible to conduct an analogous study of rhizome propagation as they were too large to feasibly study the necessary number of replicate individuals used in the study of seeds. Additionally, there was insufficient miscanthus material available to conduct a propagation study. For these reasons miscanthus and nettle propagation were only considered as part of the pot experiments.

4 Pot Experiments

Two pot experiments, conducted on successive growing seasons over the period of the study, aimed to establish the response of the plants to elevated metal concentrations in a soil matrix. The plants used in both experiments were: flax, miscanthus, nettle and oilseed rape. The three plant responses of primary interest were: survival in the contaminated matrix, biomass yield and tissue metal concentration. In both pot experiments, plants were exposed to each contaminant metal in isolation (Cd, Cr, Cu, Pb, Ni and Zn) and also a sewage sludge treated soil, which contained elevated levels of all the metals considered in the study. The metal concentrations used, together with the convention used to refer to these soils, is detailed in Table 4.1.

The first of the two pot experiments was conducted at high levels of metal contamination (Pot Experiment One; Section 4.1). The soils used represented contamination levels encountered in mine tailings or slag heaps, exemplified by dumps of chromium waste in and around Glasgow with surface soil chromium concentrations of 1500 μg/g (unpublished data).

The second pot experiment was conducted at contamination levels at, or close to, the Inter-departmental Committee on the Redevelopment of Contaminated Land (ICRCL) threshold trigger values for each of the six metals in the study (Pot Experiment Two; Section 4.2). These concentrations were chosen to establish whether the plant species in the study could reduce metal concentrations of marginally contaminated land to within the guideline levels. The Pot Experiment Two soil concentrations were also chosen to elucidate the growth and uptake response of the plant species in the study to soils with a less extreme metal loading than the Pot Experiment One soils.

Table 4.1 Naming conventions and corresponding total metal concentrations for the soil metal treatments used in Pot Experiment One and two (Section 2.6, 2.7).

Pot Experiment	Soil metal treatment	Abbreviation	Soil total metal concentration (μg/g)
One (T1 & T2 soils)	low cadmium	Cd_{T1}	326
	high cadmium	Cd_{T2}	1067
	chromium*	Cr_{T1}	2757
	low copper	Cu_{T1}	545
	high copper	Cu_{T2}	2250
	lead*	Pb_{T1}	2937
	low nickel	Ni_{T1}	729
	high nickel	Ni_{T2}	1,348
	low zinc	Zn_{T1}	731
	high zinc	Zn_{T2}	2940
Two (T3 & T4 soils)	low cadmium	Cd_{T3}	3.53
	high cadmium	Cd_{T4}	4.64
	low chromium	Cr_{T3}	829
	high chromium	Cr_{T4}	846
	low copper	Cu_{T3}	124
	high copper	Cu_{T4}	131
	low lead	Pb_{T3}	544
	high lead	Pb_{T4}	619
	low nickel	Ni_{T3}	90
	high nickel	Ni_{T4}	103
	low zinc	Zn_{T3}	282
	high zinc	Zn_{T4}	330

* In Pot Experiment One Cr and Pb were used at one concentration only.

4.1 Pot Experiment One

Elucidating plant responses to highly metal contaminated soil matrices

The aim of Pot Experiment One was to study the survival of the plants and their metal uptake in soils containing the six metals in the study (Cd, Cr, Cu, Pb, Ni and Zn) at values representing a highly contaminated matrix. Each metal in the study was represented at either one or two concentrations in Pot Experiment One (Table 4.1). Chromium and lead were studied at one concentration only as they were considered unlikely candidate metals for phytoremediation when the soils were initially produced (McGregor, 1999; Section 2.6.1.1). Having artificial soils which were prepared three years in advance of the study allowed time for the metal salts come to equilibrium with the soil the other soil constituents and so more closely resemble real contaminated soils.

4.1.1 Soil metal concentrations

Extracting solutions of increasing strength were used to give an indication of the plant availability of the soil metals (Tables 4.5–4.7). The Pot Experiment One soils were extracted using calcium chloride ($CaCl_2$), EDTA and aqua regia (Section 2.12.2, 2.12.3).

The $CaCl_2$-extractable fraction represented the most available metal fraction in the soil; metal ions in this fraction were present in the soil solution or held on weak exchange sites. The EDTA-extractable fraction represented soil metal ions held on strong exchange sites or organically bound metal ions, some of this fraction was likely to be available to plants over the period of a growing season, particularly in the presence of root exudates and soils rich in soluble organic ligands. Aqua regia was considered efficient in extracting the metal from the soil matrix and henceforth is referred to as the soil total metal concentration. This soil metal fraction will include some metal unavailable to plants over the growing season.

Expressing the $CaCl_2$- and EDTA-extractable concentration as a percentage of the soil total metal concentration facilitated visualisation of the proportion of the soil metal loading available to the plants (Tables 4.3, 4.6 and 4.9). Expressing these data in this way also allowed comparison of the mobility of each of the metals relative to each other. From this information an order of relative mobility for all the metals was produced (Table 4.4, 4.7, 4.10).

4.1.1.1 Control soil

In the control soil, Zn and Ni were the only metals present at high enough concentrations in the $CaCl_2$-extractable fraction to be detected by AAS (Table 4.2). The control soil EDTA-extractable fraction also had low metal concentrations with the exception of Pb. The control soil total metal concentrations were below the ICRCL threshold trigger values for each of the metals in the study (Section 2.7; Table 2.8), although the total Pb concentration was elevated above anticipated normal values (Ross, 1994; SEPA, 2001).

Table 4.2 Control soil metal concentrations. Standard deviations are shown (*st. dev.*); n = 4. Values below the limit of detection are denoted L.D.

Metal	$CaCl_2$-extractable		EDTA-extractable		Soil total metal conc.	
	(µg/g)	*st. dev.*	(µg/g)	*st. dev.*	(µg/g)	*st. dev.*
Cd	L.D.		0.93	*0.81*	1.42	*0.94*
Cr	L.D.		1.05	*0.10*	25	*1.54*
Cu	L.D.		14	*1.22*	35	*3.77*
Pb	L.D.		122	*14*	213	*16*
Ni	0.64	*0.47*	4.34	*0.21*	31	*1.63*
Zn	6.00	*0.42*	39	*2.86*	130	*7.54*

Zinc, the metal present in the control soil at the highest soil total concentration (Table 4.2), was the most mobile metal in the $CaCl_2$–extractable fraction (Table 4.3, 4.4). The most mobile metal in the EDTA–extractable fraction was Cd (69% of the soil total concentration). Lead was readily mobilised by EDTA with more than half the soil total Pb concentration present in the EDTA-extractable fraction (Table 4.3).

Table 4.3 Control soil metal concentrations expressed as a percentage of the soil total metal concentration.

Metal	$CaCl_2$-extractable (%)	EDTA-extractable (%)	Soil total metal conc. (µg/g)
Cd	0	65.49	1.42
Cr	0	4.20	25
Cu	0	40.00	35
Pb	0	57.28	213
Ni	2.06	14.00	31
Zn	4.62	30.00	130

The decreasing orders of mobility for the control in the $CaCl_2$- and EDTA-extractable fractions, relative to the soil total metal concentration, are shown in Table 4.4.

Table 4.4 Order of decreasing metal mobility in Pot Experiment One control soils.

Extracting solution	*Order of decreasing metal mobility*
$CaCl_2$	Zn > Ni ≫ Cd = Cu = Pb = Cr
EDTA	Cd > Pb > Cu > Zn ≫ Ni ≫ Cr

4.1.1.2 T1 And T2 artificial soils

In each of the artificial soils (Table 4.5) the metals were present above both ICRCL threshold trigger values (Section 2.7; Table 2.8) and levels accepted as phytotoxic.

Table 4.5 T1 and T2 soil metal concentrations. Values presented are mg of metal extracted per kg of dried, ground and sieved soils (Section 2.12.1.1). Standard deviations are shown (*st. dev.*); n = 4.

Treatment	$CaCl_2$-extractable		EDTA-extractable		Soil total metal conc.	
	(µg/g)	*st. dev.*	(µg/g)	*st. dev.*	(µg/g)	*st. dev.*
Cd_{T1}	129	*10*	314	*55*	326	*46*
Cd_{T2}	537	*58*	1,231	*49*	1,067	*372*
Cr_{T1}	12	*4*	48	*12*	2,757	*441*
Cu_{T1}	8	*1*	445	*84*	545	*86*
Cu_{T2}	171	*10*	1,844	*66*	2,250	*71*
Pb_{T1}	13	*0*	248	*23*	2,937	*59*
Ni_{T1}	254	*10*	560	*23*	729	*38*
Ni_{T2}	640	*125*	1,089	*142*	1,348	*137*
Zn_{T1}	215	*13*	558	*46*	731	*63*
Zn_{T2}	1,534	*137*	2,856	*200*	2,940	*234*

In the $CaCl_2$-extractable fraction Cd_{T1}, Cd_{T2}, Ni_{T1}, Ni_{T2}, Zn_{T1} and Zn_{T2} were present at concentrations of 25–50% of the soil total metal concentration (Table 4.6); Cr_{T1} and Pb_{T1} were the least mobile in the $CaCl_2$-extractable fraction, present at <0.5% of the soil total metal concentration. In the $CaCl_2$-extractable fraction, Cu_{T1} and Cu_{T2} were present at 1.5–7.6% of the soil total metal concentration and so had an intermediate mobility with relation to the other metals. The solubility of the soil metals was much more consistent between metal species in the EDTA-extractable fraction (Table 4.6). With the exception of Cr_{T1}, the metals in the study were present in the EDTA-extractable fraction at 76–100% of the soil total metal concentration. $Chromium_{T1}$, present in the EDTA-extractable fraction at <2% of the soil total metal concentration, was not considered available to plants over the growing season; this level of Cr mobility has been reported for other soils (Neale *et al.*, 1997).

Table 4.6 Soil extracts expressed as a percentage of the soil total metal concentration.

Treatment	$CaCl_2$-extractable (%)	EDTA-extractable (%)	Soil total metal conc. (μg/g)
Cd_{T1}	40	96	326
Cd_{T2}	50	115	1,067
Cr_{T1}	0.44	1.7	2,757
Cu_{T1}	1.5	82	545
Cu_{T2}	7.6	82	2,250
Pb_{T1}	0.44	85	2,937
Ni_{T1}	35	77	729
Ni_{T2}	48	81	1,348
Zn_{T1}	29	76	731
Zn_{T2}	52	97	2,940

The decreasing orders of metal mobility in the T1 and T2 soils for both $CaCl_2$- and EDTA-extractable fractions are summarised in Table 4.7.

Table 4.7 Order of decreasing metal mobility in the T1 and T2 soils.

Extracting solution	*Order of decreasing metal mobility*
$CaCl_2$	$Cd \geq Zn \geq Ni \gg Cu > Pb = Cr$
EDTA	$Cd > Zn > Pb > Cu > Ni \gg Cr$

4.1.1.3 Sewage sludge soil

Metal concentrations in the sewage sludge soil were above the ICRCL threshold trigger values (Section 2.7; Table 2.8) for all of the metals in the study (Table 4.8). The sewage sludge Cu, Ni and Zn soil concentrations were also higher than the Ni_{T1} and Zn_{T1} soil concentrations (Table 4.5, 4.8). The $CaCl_2$-extractable fraction of the sewage sludge soil had little or no detectable metal present for each of the metals in the study (Table 4.8); the highest $CaCl_2$-extractable metal concentration was Ni, present at 4.80 μg/g, representing only 1.11% of the soil total Ni concentration (Table 4.9). Considering the $CaCl_2$-extractable fraction as a percentage of the soil total metal concentration, Cd, Cu, Ni and Zn were markedly less mobile than in the corresponding T1 and T2 soils (Table 4.6, 4.9). Since all metals were present at <1.2% of the soil total metal concentration, the availability of metals in the sewage sludge soil's most mobile fraction was comparable to the availability of Pb and Cr, the least mobile metals, in the T1 and T2 soils (Table 4.6, 4.9). The low mobility of the metals in the sewage sludge soil was likely due to the high organic matter content of the sewage sludge soil.

Table 4.8 Sewage sludge soil metal concentrations. Standard deviations are shown (*st. dev.*); n = 4. Values below the limit of detection are denoted L.D.

Metal	$CaCl_2$-extractable		EDTA-extractable		Soil total metal conc.	
	(µg/g)	*st. dev.*	(µg/g)	*st. dev.*	(µg/g)	*st. dev.*
Cd	L.D.		23	*10*	41	*3.67*
Cr	L.D.		3.84	*0.45*	1,962	*148*
Cu	1.42	*0.18*	550	*25*	881	*46*
Pb	L.D.		287	*25*	695	*33*
Ni	4.80	*0.17*	174	*13*	431	*24*
Zn	2.49	*0.50*	1,075	*63*	2,126	*157*

Table 4.9 Sewage sludge soil metal concentrations expressed as a percentage of the soil total metal concentration.

Metal	$CaCl_2$-extractable	EDTA-extractable	Soil total metal conc.	
	(%)	(%)	(µg/g)	*st. dev.*
Cd	0	56.10	41	*3.67*
Cr	0	0.20	1,962	*148*
Cu	0.16	62.43	881	*46*
Pb	0	41.29	695	*33*
Ni	1.11	40.37	431	*24*
Zn	0.12	50.56	2,126	*157*

The EDTA-extractable fraction of the sewage sludge soil, like that of the $CaCl_2$-extractable fraction, contained less metal relative to the soil total metal concentration than the corresponding metals in the T1 and T2 soils. On average, EDTA extracted 37% less metal from the sewage sludge soil than from the T1 and T2 soils. The difference in relative mobility between the T1 and T2 soils (Table 4.7) and the sewage sludge soils (Table 4.10) was metal dependant. Differing soil total metal concentrations prevented direct comparison of the EDTA-extractable concentrations between the sewage sludge soil and the T1 and T2 soils, however, the orders of relative mobility could be compared.

Table 4.10 Order of decreasing metal mobility in Pot Experiment One sewage sludge soil.

Extracting solution	*Order of decreasing metal mobility*
$CaCl_2$	Ni > Cu > Zn > Cd = Pb = Cr
EDTA	Cu > Cd > Zn > Pb > Ni ≫ Cr

Comparison between the orders of mobility of the T1 and T2 soils (Table 4.7) and the sewage sludge soil (Table 4.10) indicated that in the $CaCl_2$-extractable fraction Ni replaced Cd as the most mobile metal, however, the percentage of $CaCl_2$-extractable metal was low.

From the T1 and T2 soils to the sewage sludge soil, the relative mobility of Zn decreased such that Zn became the least mobile metal present above the limit of detection. Cadmium, the most mobile metal in the T1 and T2 soils $CaCl_2$-extractable fraction, was not detected in the sewage sludge soil $CaCl_2$-extractable fraction.

The only change in the order of mobility in the EDTA-extractable fraction, from T1 and T2 soils to the sewage sludge soil, was the relative mobility of copper; Cu, the fourth most mobile metal in the T1 and T2 soils, became the most mobile metal in the sewage sludge soil. All of the metals had a lower EDTA-extractable concentration in the sewage sludge soil than in the T1 and T2 soils. The reduction in the mobility of Cu, however, was less than the reduction in the mobility of Cd, Pb, Ni, and Zn, with the result that Cu was the most mobile metal in the sewage sludge soil.

4.1.2 Plant Growth

The survival, height and yield of each plant species in the study was considered (Section 2.6). The plant species investigated were: flax, miscanthus, nettle and oilseed rape. No oilseed rape plants survived in the T1 and T2 soils and thus no further reference will be made to oilseed rape in relation to Pot Experiment One.

4.1.2.1 Survival and height data

Different phenotypic characteristics between plant species dictated that only intra-species comparisons of survival and height data be made.

4.1.2.1.1 Flax

Flax survival

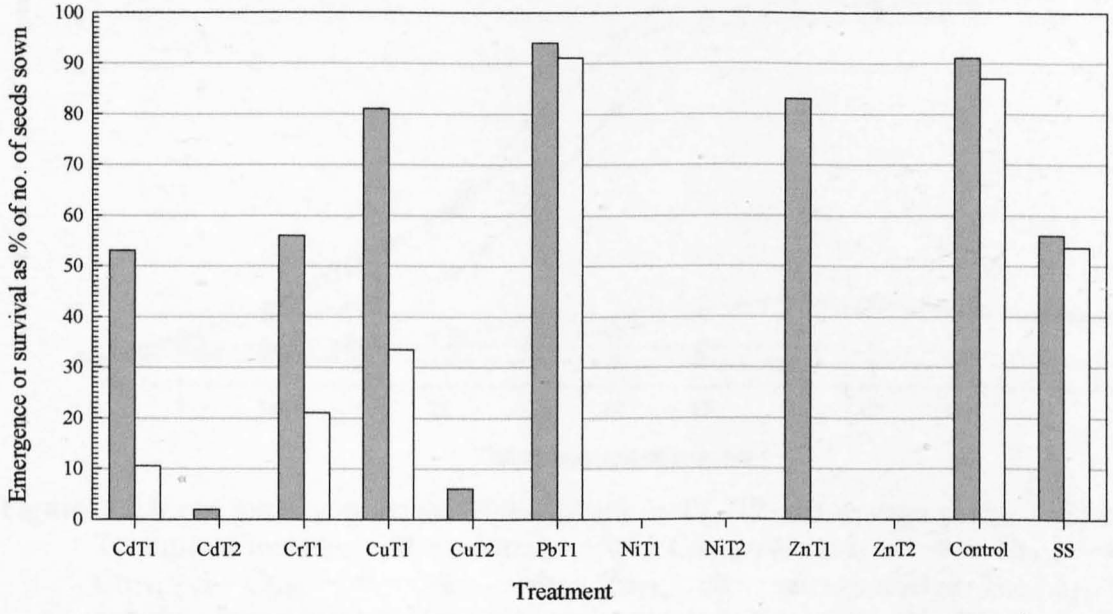

Figure 4.1 Emergence and survival of flax shoots in T1, T2 and sewage sludge (SS) soils. The total number of flax plants emerging (■) and surviving until the end of the growth period (□) are expressed as a percentage of the number of seeds sown.

The survival of the flax plants in the highly contaminated environment of the T1 and T2 soils was poor (Fig. 4.1). In the Ni_{T1}, Ni_{T2} and Zn_{T2} soils, there was no flax plant germination. In the Cd_{T2}, Cu_{T2} and Zn_{T1} soils, all the plants which initially germinated did not survive until the end of the growth period; of these treatments Zn_{T1} gave an initial germination of over 80%, whereas the Cd_{T2}, Cu_{T2} treatments exhibited germination of <10%. Control and Pb_{T1} soils were the only treatments which gave a greater germination than the Zn_{T1} soil. The germination in the Cd_{T1}, Cr_{T1} and Cu_{T1} soils was 53%, 56% and 81%, respectively, however, the number of plants surviving at the end of the growth period was <⅓ of the number of seeds sown. Only the control, Pb_{T1}, and sewage sludge soils had

within 10% of the number of germinated plants surviving until the end of the growth period. In each of these treatments >50% of the seeds sown germinated, with Pb exhibiting the highest final number of surviving plants of all the flax–metal treatments.

Flax height

The height data for the flax plants (Fig. 4.2) supported the survival data. Again the control, sewage sludge and Pb_{T1} treatments produced the best growth, with mean maximum heights >70 cm. $Chromium_{T1}$ and Cu_{T1} were the only other soil treatments to produce plants with final maximum mean heights significantly >0 cm, at mean heights of approximately 10 cm.

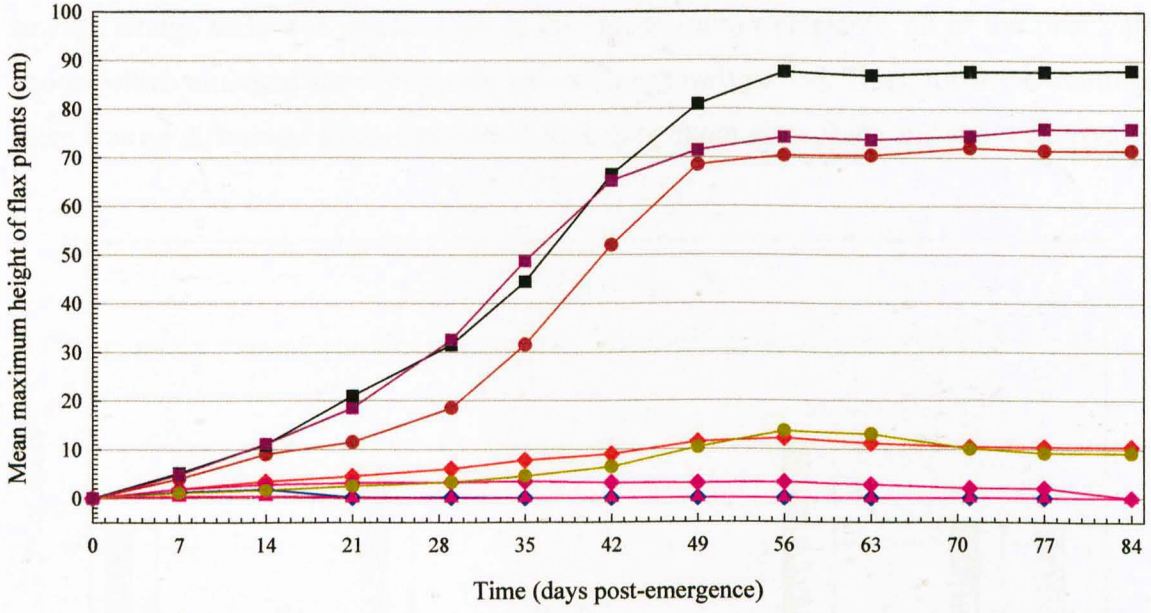

Figure 4.2 Mean maximum height of flax plants in T1, T2 and sewage sludge (SS) soils. Treatments include: control, Cd_{T1}, Cd_{T2}, Cr_{T1}, Cu_{T1}, Cu_{T2}, Pb_{T1}, Zn_{T1}, sewage sludge. The Ni_{T1}, Ni_{T2} and Zn_{T2} treatments have been omitted as plants in these soils did not germinate.

4.1.2.1.2 Miscanthus

Miscanthus survival

The survival of the miscanthus plants in the T1 and T2 soils (Fig. 4.3) was better than the survival of the flax plants (Fig. 4.2) relative to the their respective control plants. Unlike flax which was sown from seed, miscanthus was planted as pieces of rhizome material. Although the rhizomes planted were of a uniform size with an equal number of nodes, the final number of shoots produced by each rhizome, if any, was unpredictable. The greatest number of shoots was produced by plants in Zn_{T1} and Cu_{T1} soils at 15 and 14 shoots, respectively, not in the control soil, which, like the sewage sludge and Cd_{T1} soils, produced 11 shoots. The tolerance of the emerging miscanthus plants to the Cd_{T1}, Pb_{T1}, Zn_{T1} and sewage sludge soils was greater than in flax as, in these treatments, all of the miscanthus shoots which emerged survived to the end of the growth period. Thus, for these treatments there was no difference in the toxicity of the soil to shoot emergence and shoot survival.

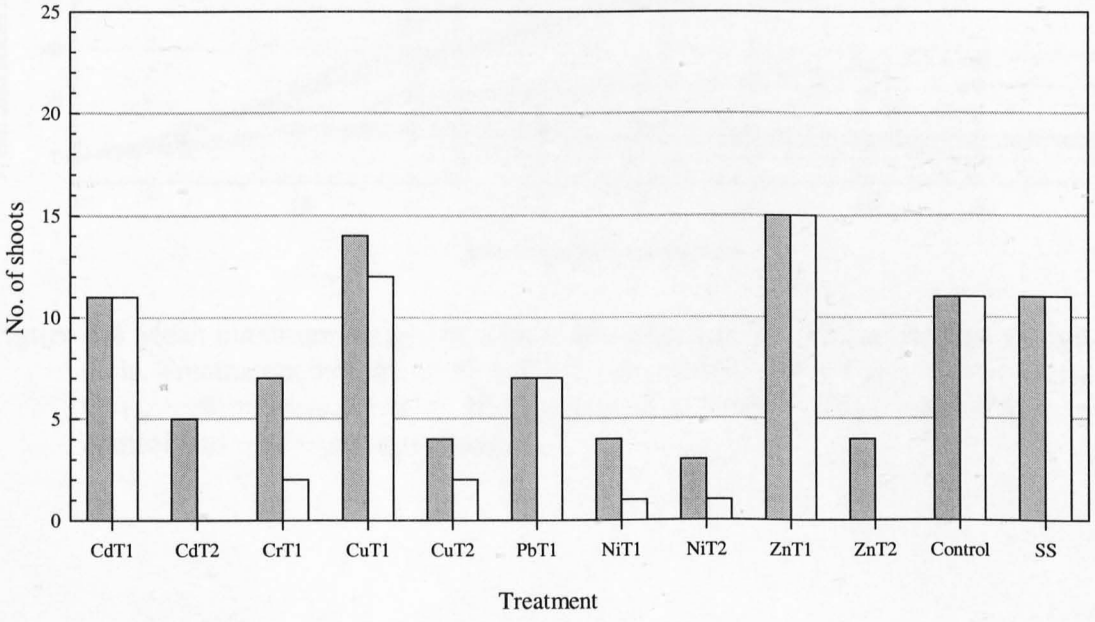

Figure 4.3 Emergence and survival of miscanthus shoots in T1, T2 and sewage sludge (SS) soils. Values shown are the total number of miscanthus shoots emerging (■) and surviving until the end of the growth period (□). Emergence and survival have been expressed in numbers of shoots rather than a % (Fig. 4.1) since maximum shoot emergence could not be predicted.

None of the treatments was sufficiently toxic to prevent miscanthus shoot emergence as all treatments produced some miscanthus shoots. In the case of the Cd_{T2} and Zn_{T2} soils, however, none of the plants survived to the end of the growth period (Fig. 4.3). Of the treatments where plants survived until the end of the growing period, the poorest treatments were Ni_{T1} and Ni_{T2} each with only one shoot surviving. In both the Cd_{T2} and Cr_{T1} soils, five of the emerging shoots subsequently died.

Miscanthus height

The control shoots grew to a mean maximum height of 160 cm and were >50% taller than the next tallest treatment's shoots (Fig. 4.4). The Pb_{T1} and Zn_{T1} miscanthus shoots were the tallest of the T1 and T2 miscanthus shoots followed by sewage sludge soil; these treatments had shoot heights of 90, 87 and 70 cm respectively. The Cu_{T1} and Cd_{T1} produced plants which were stunted at a mean height of up to 40 cm. Several of the miscanthus treatments produced plant mean shoot heights <21 cm, these were Cd_{T2}, Cr_{T1}, Cu_{T2}, Ni_{T1} Ni_{T2} and Zn_{T2}.

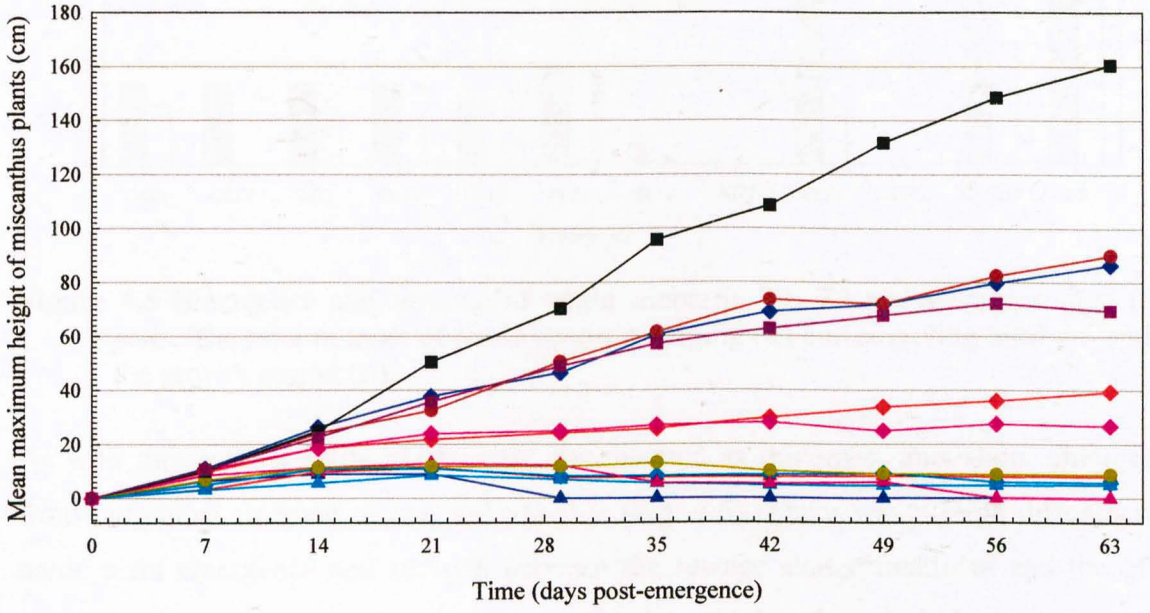

Figure 4.4 Mean maximum height of miscanthus plants in T1, T2 and sewage sludge (SS) soils. Treatments include: Cd_{T1}, Cd_{T2}, Cr_{T1}, Cu_{T1}, Cu_{T2}, Pb_{T1}, Ni_{T1}, Ni_{T2}, Zn_{T1}, Zn_{T2}, control and sewage sludge.

4.1.2.1.3 Nettle

Nettle survival

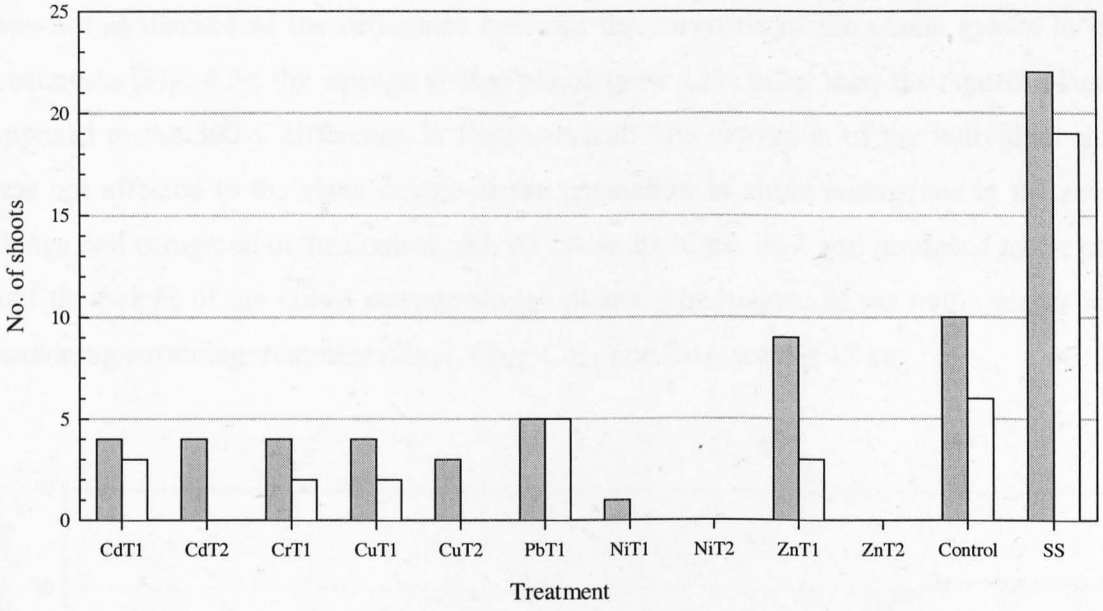

Figure 4.5 Emergence and survival of nettle shoots in T1, T2 and sewage sludge (SS) soils. The total number of nettle shoots emerging (■) and surviving until the end of the growth period (□).

As with miscanthus, nettle plants were also planted as rhizomes, thus shoot emergence from individual rhizome pieces was variable (Fig. 4.5). There was a large difference in nettle plant emergence and survival between the sewage sludge treatment and the other treatments in the experiment with emergence and survival in sewage sludge soil more than double that of the control. Of the 22 sewage sludge shoots that emerged, 19 survived to the end of the growth period, three times the number that survived in the control soil. $Zinc_{T1}$ produced a total of nine shoots only three of which survived the duration of the growth period. $Lead_{T1}$, which produced five shoots, was the only treatment with all emerging shoots surviving to the end of the growth period. Only two treatments, Ni_{T2} and Zn_{T2}, failed to produce any shoots at all. There were three treatments where shoots did emerge but died before the end of the growth period; these were Cd_{T2}, Cu_{T2} and Ni_{T1}. As with flax no plants survived in either of the Ni treated soils.

Nettle height

The difference between the heights of the sewage sludge and the control plants (Fig. 4.6) was not as marked as the difference between the survivals of the plants grown in these treatments (Fig. 4.5); the sewage sludge plants grew 12% taller than the control plants as opposed to the 300% difference in final survival. The extension of the individual shoots was not affected to the same degree as the promotion of shoot emergence in the sewage sludge soil compared to the control soil. At 21 cm high, the Pb_{T1} soil produced nettle plants half the height of the tallest sewage sludge plants. The heights of the nettle plants in the remaining surviving treatments Zn_{T1}, Cr_{T1}, Cd_{T1} and Cu_{T1} were $\leq$ 12 cm.

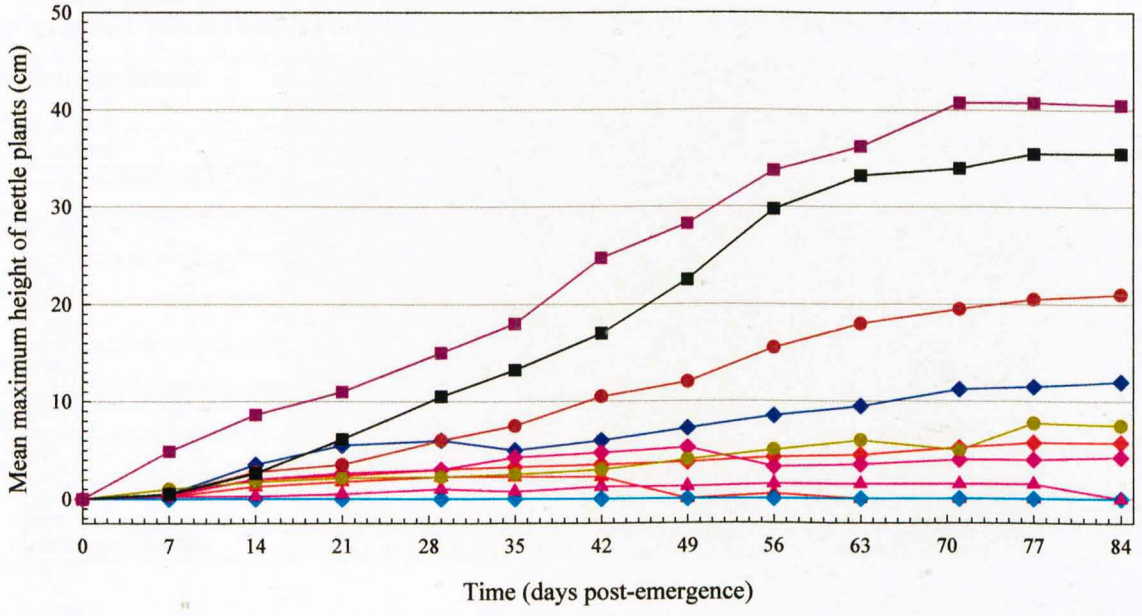

Figure 4.6 Mean maximum height of nettle plants in T1, T2 and sewage sludge (SS) soils. Treatments include: control, Cd_{T1}, Cd_{T2}, Cr_{T1}, Cu_{T1}, Cu_{T2}, Pb_{T1}, Ni_{T1}, Zn_{T1} and sewage sludge. The Ni_{T2} and Zn_{T2} treatments have been omitted as plants in these soils did not emerge.

4.1.2.2 Yield data

Plant yields from each treatment within plant species were expressed as a percentage of that plant species control biomass (Table 4.11), allowing inter-species comparison of yield response to the metal treatments. In this section the % yields discussed refer to the treatment plant biomass as a percentage of control treatment plant biomass. The Cd_{T2}, Cu_{T2} and Zn_{T2} soils did not yield sufficient plant biomass (< 0.15 g) to allow yields to be calculated for any of the plants in the study, no further reference will be made to these treatments in this section.

Table 4.11 Fresh weight yield of above ground plant tissue.

Yield of plants grown in T1 and T2 soils expressed as a percentage of the control plant biomass. Yields are calculated from the mean of four replicate pots (n=4). Where n<4 this is indicated by: † n=3, ‡ n=2, ⊥ n=1.

Soil Treatment	Flax		Miscanthus		Nettle	
	(g)	*st. dev.*	(g)	*st. dev.*	(g)	*st. dev.*
Control	51.85	*6.88*	59.65	*35.26*	9.99	*2.50* †
	(%)	*st. dev.*	(%)	*st. dev.*	(%)	*st. dev.*
Cd_{T1}	0	*0*	6	*4.12* †	10.43	⊥
Cr_{T1}	0.5	⊥	0.4	⊥	50	⊥
Cu_{T1}	3	*2.10* ‡	10	*10.78*	63	⊥
Pb_{T1}	69	*0.79* ‡	92	*67.47* ‡	84	⊥
Ni_{T1}	0	*0*	0.3	⊥	0	*0*
Ni_{T2}	0	*0*	2	⊥	0	*0*
Zn_{T1}	0	*0*	72	*23.35*	34	*84.15* ‡
Sewage Sludge	66	*29.20*	26	*11.83*	386	*63.52*

Flax yield

The control soil yielded the greatest above ground biomass producing approximately 52 g of material (Table 4.11). The highest yielding plants in the T1 and T2 soils grew in the Pd_{T1} soil with a biomass of 69%. The sewage sludge soil yield was 66%, despite the sewage sludge soil plants, on average, being taller than the Pd_{T1} soil plants. This reduction in yield was due to a higher flax germination in the Pd_{T1} soil (Fig. 4.1). The other T1 and T2 soils gave low biomass yields with Cu_{T1} and Cr_{T1} yielding only 3% and 0.5%, respectively.

Miscanthus yield

The miscanthus plants grown in the control soil yielded the greatest above ground biomass (~60 g) of all the soil–plant systems in Pot Experiment One. For the treated soils, the miscanthus yield data (Table 4.11) was broadly in agreement with the height and survival data (Section 4.1.2.1.2). The plants grown in the Pb_{T1} soil had the greatest yield at 92%. The Zn_{T1} plants, which despite having both a greater maximum mean height and more shoots than Pb_{T1} soil, had a lower yield (72%). The sewage sludge soil gave a plant yield of 26% followed by the Cu_{T1} and Cd_{T1} which gave yields of 10% and 6%, respectively. The poorest plant yields for treatments with plants surviving until the end of the growth period, all of which were <5%, were from the Ni_{T1}, Ni_{T2} and Cr_{T1} soils.

Nettle yield

The most notable feature of the yield data for the nettle plants was the extent to which the sewage sludge plants outgrew the other treatments including the control plants (Table. 4.11). The yield response of nettles grown in the sewage sludge soil compared to the plants grown in the control soil supported the observed shoot emergence response (Section 4.1.2.1.3); this indicated that the height data, which was not markedly different for the control and sewage sludge soils, was not a true reflection of the plants' ability to produce biomass. The Pb_{T1} yield of 84% also supported the similarity between yield response and shoot survival, as the survival of the Pb_{T1} shoots was 83% of the control shoots survival.

Nettle yields in the Cu, Cr, Zn and Cd soils were better relative to the control soil than the miscanthus and flax yields (Table 4.11). Given the yield response in sewage sludge soil, nettle was the species which showed the greatest potential for improving growth by incorporation of fertiliser into the remediation strategy.

4.1.2.3 Growth summary

Miscanthus plants performed well in most of the soil treatments in terms of emergence relative to the control plants. The control miscanthus plant height of 160 cm observed for the control plants was lower than heights reported by Bullard and Kilpatrick (1997). Post-emergence, survival rates for miscanthus were also better than the other plant species as in five out of 12 treatments (Cd_{T1}, Pb_{T1} Zn_{T1}, control and sewage sludge) all of the emerging plants survived. The only other plant species–treatment where all the emerging plants survived to the end of the growth period was the nettle–Pb_{T1} treatment. Miscanthus was also the highest yielding plant species, however, the growth of the plants was markedly affected by the metal treatments with only the Pb_{T1}, Zn_{T1} and sewage sludge treatments yielding biomass >25% of the control biomass. In contrast to miscanthus, flax growth was poor in most of the T1 and T2 soils, the exceptions being the Pb_{T1} and sewage sludge treatments. Flax, after oilseed rape, was the least tolerant plant species to the T1 and T2 soils with Ni proving to be the most toxic followed by Zn.

The ability of the flax plants to germinate in the soil was not directly related to their ability to survive in that soil (Section 3); for instance, Zn_{T1} allowed good initial germination but did not have any seedlings surviving to the end of the growth period. Soil toxicity to the flax plants was manifest in two distinct ways: prevention of seedling emergence (Ni_{T1}), or post emergence seedling death (Zn_{T1}). These toxic effects were also observed in the nettle and miscanthus plants although to a lesser extent.

Each plant species performed well in the sewage sludge soil despite it's high metal loading. In particular the nettle plants grown in the sewage sludge soil yielded ~ four fold more biomass than the control plants. This was likely attributable to nettles' ability to thrive in fertile soils (Grieve, 1931). Furthermore, flax plants did not survive in the Zn_{T1} soil yet the flax growth performance in the sewage sludge soil, which had a higher soil Zn concentration than Zn_{T1} soil, was comparable to that of the control. Thus, both the availability of the soil metals to the plants and the fertility of the soil are fundamental in understanding growth of plants in contaminated soils. It is also necessary to consider the suitability of the plants to the growth conditions of the contaminated matrix as this has major implications for the use of nettles in phytoremediation. Many contaminated soils are low in nutrients, in which nettles will not thrive, however, it may be possible to overcome this problem by the addition of a fertiliser treatment or the inclusion of a soil amendment such as sewage sludge in the remediation strategy. This strategy may significantly improve the ability of nettle to act as a phytpremediator.

The poor growth response of the plants in the study to the T4 soils broadly agreed with the observed survival rates of tree species grown in the same soils in an earlier study (McGregor, 1999); the tree species also had poor survivals in the T4 soils after 10 months growth.

4.1.3 Plant tissue metal concentrations

To elucidate the plant species' potential as effective phytoremediators it was essential to measure their tissue metal concentrations. The plant tissue metal concentrations (μg/g) for each plant species are shown in Tables 4.12–4.14. For the T1 and T2 soils, these values were also expressed as a percentage of the soil total metal concentration (Fig. 4.7–4.12) allowing visualisation of metal uptake and metal mobility in the soil–plant system. Flax stem and leaf tissues were analysed together and in addition flax roots were analysed. The stem and leaf tissues of miscanthus and nettle were analysed separately, however, root tissues were not analysed (Section 2.6.2).

4.1.3.1 Control soil

Table 4.12 Tissue metal concentrations of plants grown in the control soil. Mean plant tissue metal concentrations (μg/g) for: (a) flax, (b) miscanthus and (c) nettle. Values are the average of four replicate pots (n=4) except for (c) nettle where n=3. Where the values are below the limit of detection these are indicated by L.D.

(a) Flax	Cd		Cr		Cu	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Roots	5.76	*1.28*	L.D.		8.94	*4.86*
Stem + Leaf	3.36	*0.16*	L.D.		6.90	*5.55*
	Pb		Ni		Zn	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Roots	158.47	*305.51*	0.74	*1.48*	84.21	*22.15*
Stem + Leaf	21.69	*43.38*	0.25	*0.50*	59.73	*13.37*

(b) Miscanthus	Cd		Cr		Cu	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Stem	L.D.		2.10	*0.57*	5.87	*1.27*
Leaf	0.50	*0.99*	3.26	*1.45*	3.31	*0.46*
	Pb		Ni		Zn	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Stem	L.D.		2.11	*0.70*	101.92	*45.53*
Leaf	L.D.		2.95	*0.56*	43.37	*15.41*

(c) Nettle	Cd		Cr		Cu	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Stem	0.69	*1.20*	L.D.		3.53	*1.06*
Leaf	0.81	*1.40*	L.D.		9.54	*5.36*
	Pb		Ni		Zn	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Stem	L.D.		5.47	*0.83*	114.48	*42.75*
Leaf	11.40	*0.02*	7.05	*2.84*	191.82	*65.01*

Flax

In the control soils all of the metals measured that were above detectable levels, were present at higher concentrations in the root tissue than in the above ground (stem + leaf) tissues (Table 4.12a). Lead was taken up in the greatest concentration in root tissue but had the greatest reduction in tissue concentration from root to above ground tissue (root to shoot ratio of 7:1). In the above ground tissue, Zn was present at the highest tissue metal concentration (59 μg/g). Chromium was not detected in flax tissues, and above ground tissue concentrations of both Ni and Cu were low.

Miscanthus

Zinc was present at the highest tissue metal concentration in the control soils (Table 4.12b). The miscanthus stems contained more Zn than the miscanthus leaves (102 μg/g and 43 μg/g, respectively). Lead, despite being present in the control soil at the highest soil metal concentration (Table 4.2), was not detected in the miscanthus tissues. The other metals were present in the miscanthus tissues at low concentrations.

Nettle

The distribution of Zn in the tissues of nettle plants grown in the control soil differed from that of miscanthus in that nettles had a higher tissue concentration in the leaves (192 μg/g) than nettle shoots (114 μg/g). Like miscanthus and flax, Zn was also taken up in above ground tissue of nettle at the highest concentration of all the metals. Chromium was the only metal which was not detected in the nettle–control plant tissues, uptake of the remaining metals into the nettle tissue was low.

4.1.3.2 T1 And T2 artificial soils

Of the T1 and T2 soil treatments, Cd_{T2}, Cu_{T2} and Zn_{T2} soils did not produce sufficient plant material to allow analysis of flax, miscanthus or nettle. In the remaining soil treatments (Table 4.13): miscanthus plants produced sufficient material for analysis although it was necessary to pool the stem and leaf tissue from the Cr_{T1} and Ni_{T1} soils; nettle produced sufficient material for analysis in all but the two Ni soils; flax yielded enough plant biomass for analysis in only two of the soil treatments, Cu_{T1} and Pb_{T1}.

Table 4.13 Tissue metal concentrations of plants grown in the T1 and T2 soils. Mean plant tissue metal concentrations ($\mu g/g$) for: (a) flax, (b) miscanthus and (c) nettle. The 'n' number represents the number of replicate pots. The standard deviation is given (*st. dev.*). ✳ denotes stem + leaf tissue. Values are calculated from the mean of four replicate pots (n=4). Where n<4 this is indicated by: † n=3, ‡ n=2, ⊥ n=1.

Soil Treatment	Plant Tissue	Flax		Miscanthus		Nettle	
		($\mu g/g$)	*st. dev.*	($\mu g/g$)	*st. dev*	($\mu g/g$)	*st. dev*
Cd_{T1}	Stem	No growth		15.00	*21* ‡	918.88	⊥
	Leaf	No growth		176.00	*180* †	851.68	⊥
Cr_{T1}	Stem	No growth		55.00✳	⊥	7.79	⊥
	Leaf	No growth				17.47	⊥
Cu_{T1}	Root	175.55	*79* ‡				
	Stem	18.94✳	*1.4* ‡	7.00	*7*	4.32	⊥
	Leaf			10.00	⊥	10.97	⊥
Pb_{T1}	Root	416.53	*589* ‡				
	Stem	33.46✳	*47* ‡	52.00	*17* ‡	194.20	⊥
	Leaf			119.00	*1* ‡	111.00	⊥
Ni_{T1}	Stem	No growth		186.00✳	⊥	No growth	
	Leaf	No growth				No growth	
Ni_{T2}	Stem	No growth		4.00	⊥	No growth	
	Leaf	No growth		5.00	⊥	No growth	
Zn_{T1}	Stem	No growth		969.00	*50*	171.47	⊥
	Leaf	No growth		868.00	*109*	191.96	⊥

Flax

Flax roots grown in the Pb_{T1} soil had the highest tissue metal concentration at 417 μg/g, however, this result had a high standard deviation owing to one of the two samples analysed giving a high uptake of Pb whilst the other replicate sample yielded no detectable Pb. Uptake of Cu and Pb by the roots of the flax plants was 9 and 12 times greater than the metal transported to the above ground (stem + leaf) tissue, respectively (Table 4.13; Fig. 4.7). Lead was present in both flax root and above ground tissues at higher concentrations than Cu (Table 4.13, 4.14). However, flax above ground tissue extracted a greater proportion of the Cu from the Cu_{T1} soil than Pb from the Pb_{T1} soil in proportion to the soil metal loadings of these treatments, indicating that Cu was more mobile in the soil–flax system than Pb (Table 4.14; Fig. 4.7).

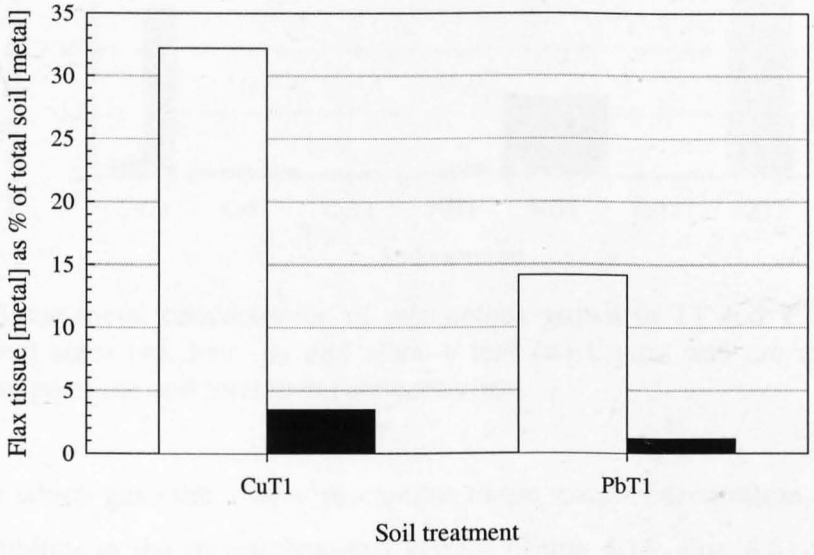

Figure 4.7 Tissue metal concentration of flax grown in T1 and T2 soils. Values, expressed as a percentage of the soil total metal concentration, represent root (□) and stem + leaf (■) tissues.

Table 4.14 Order of total metal uptake and mobility in flax tissue. Values expressed: (a) by tissue metal concentration (uptake), (b) by tissue metal concentration expressed as a percentage of the soil total metal concentration (mobility). Abbreviations used: R, root tissue; B, both stem and leaf tissue.

(a) Order of decreasing uptake
$Pb_{T1}R > Cu_{T1}R \geq Pb_{T1}B > Cu_{T1}B$

(b) Order of decreasing mobility
$Cu_{T1}R > Pb_{T1}R \geq Cu_{T1}B > Pb_{T1}B$

Miscanthus

The highest miscanthus tissue metal concentrations were recorded in the Zn_{T1} soil, where the stems were found to contain more Zn than the leaves at 969 μg/g and 868 μg/g, respectively (Table 4.13; Fig. 4.8). Zinc, the most mobile element in the soil–miscanthus system (Table 4.15), present at 133% of the Zn_{T1} soil total Zn concentration, was the only metal to be accumulated by miscanthus plants (Fig. 4.8).

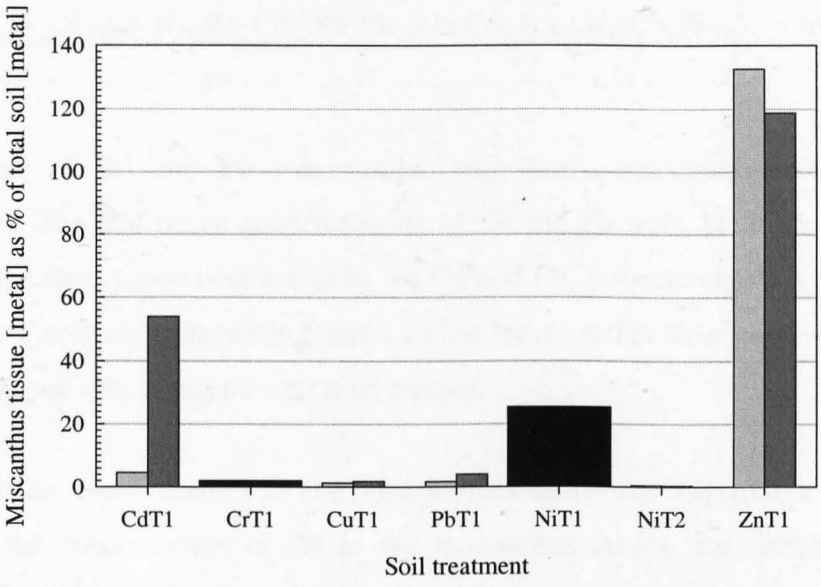

Figure 4.8 Tissue metal concentration of miscanthus grown in T1 and T2 soils. Values represent stem (▪), leaf (■) and stem + leaf (■) tissues and are expressed as a percentage of the soil total metal concentration.

The treatment which gave the lowest miscanthus tissue metal concentration, Ni_{T2} also had the lowest mobility in the miscanthus–soil system (Table 4.15; Fig. 4.8). However, the behaviour of Ni in the miscanthus plants was peculiar in that the Ni_{T1} plants had tissue concentrations 40 times greater than Ni_{T2} plants. The low Ni uptake by the single miscanthus plant surviving in the Ni_{T2} soil may be the result of a plant mechanism which prevented Ni uptake by this individual. Such a mechanism was not evident in other plants growing in either of the Ni soils (Table 4.13). The data suggested that the single miscanthus plant surviving in the Ni_{T1} soil was more representative of the miscanthus population, and that the high Ni tissue concentration in this individual, resulted from the plant having no ability to exclude Ni from its tissues, leading to poor growth and the death of it's cohorts (Section 4.1.2).

Table 4.15 Order of total metal uptake and mobility in miscanthus tissue. Values expressed: (a) by tissue metal concentration (uptake), (b) by tissue metal concentration expressed as a percentage of the soil total metal concentration (mobility). Abbreviations used: S, stem tissue; L, leaf tissue; B, both stem and leaf tissue.

(a) Order of decreasing uptake
$Zn_{T1}S > Zn_{T1}L \gg Ni_{T1}B > Cd_{T1}L > Pb_{T1}L > Cr_{T1}B \geq Pb_{T1}S > Cd_{T1}S > Cu_{T1}B > Ni_{T2}B$

(b) Order of decreasing mobility
$Zn_{T1}S > Zn_{T1}L \gg Cd_{T1}L > Ni_{T1}B > Cd_{T1}S > Pb_{T1}L \geq Cr_{T1}B > Cu_{T1}L > Pb_{T1}S > Cu_{T1}S > Ni_{T2}B$

The behaviour of Cd and Pb was similar with leaf concentration exceeding stem concentrations. The leaf tissue concentrations of Cd and Pb were 11 times and 2.3 times greater than the stem tissue concentration for Cd and Pb, respectively, this suggested that the plants were actively transporting metal to the leaves rather than passively depositing metal on exchange sites along transpiration stream.

Although the Zn concentration in the miscanthus stems was approximately 100 μg/g greater than the concentration of Zn in the miscanthus leaves, the difference was not significant (*t* test, $P>0.05$). Thus the similarity in concentration between the tissues of miscanthus indicated that the Zn was neither transported preferentially to the leaves nor the stems of the miscanthus plants. In Cu_{T1} and Ni_{T2} the metals within the stem and leaf tissues were also present at similar concentrations therefore like Zn there was no indication of a mechanism for preferential deposition of these metals in either tissue. This does not necessarily imply the same transport mechanism was operating for all three metals as the concentration of Zn in the plant tissues was two orders of magnitude greater than the tissue concentrations of Cu and Ni.

Nettle

In nettle, like miscanthus, the metal present at the highest tissue metal concentrations was also the most mobile metal in the soil–plant system, however, for nettle this metal was Cd (Tables 4.13 and 4.16; Fig. 4.9). Cadmium was the only metal in the T1 and T2 soils to be accumulated by nettle. Conversely Cr and Cu were present in nettle tissues at the lowest tissue concentrations, and were the least mobile metals in the soil–nettle system. Mean tissue Cd concentrations were 18 times higher in the nettle–soil system than in the miscanthus–soil system, however, the reverse was true for Zn, which had a mean nettle tissue concentrations five times lower than miscanthus.

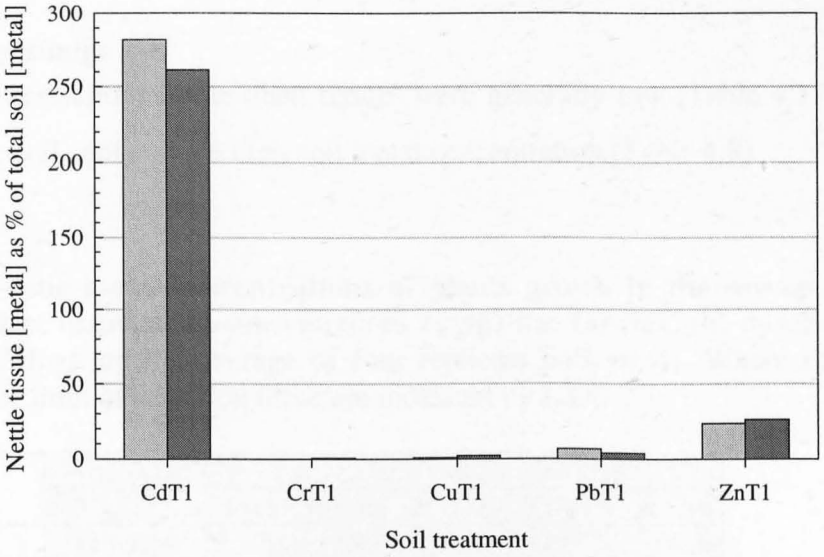

Figure 4.9 Tissue metal concentration of nettle grown in T1 and T2 soils. Values represent stem (▒) and leaf (■) tissues and are expressed as a percentage of the soil total metal concentration.

Table 4.16 Order of total metal uptake and mobility in nettle tissue. Values expressed: (a) by tissue metal concentration (uptake), (b) by tissue metal concentration expressed as a percentage of the soil total metal concentration (mobility). Abbreviations used: S, stem tissue; L, leaf tissue.

(a) Order of decreasing uptake
$Cd_{T1}S > Cd_{T1}L \gg Pb_{T1}S \geq Zn_{T1}L > Zn_{T1}S > Pb_{T1}L \gg Cr_{T1}L > Cu_{T1}S > Cu_{T1}L > Cr_{T1}S$
(b) Order of decreasing mobility
$Cd_{T1}S > Cd_{T1}L \gg Zn_{T1}L > Zn_{T1}S > Pb_{T1}S > Pb_{T1}L > Cu_{T1}S > Cu_{T1}L > Cr_{T1}L > Cr_{T1}S$

In the Cu_{T1}, Cr_{T1} and Pb_{T1} nettle treatments there were a low number of surviving replicates, however, the data from the surviving plants was interesting as the distribution of metals between tissues in nettle contrasted with that of miscanthus. Copper and Cr were present in the nettle leaf tissue at higher concentrations than in the nettle stem tissue whereas in miscanthus these metals were present in both tissues at similar levels. These nettle tissue concentrations indicated a preferential deposition of both Cr and Cu in the leaves rather than the stems. Lead was present in the nettle stem tissue at a higher concentration than in the leaf tissue whereas in the miscanthus tissues the reverse was true.

4.1.3.3 Sewage sludge soil

The metal concentrations in the plant tissues were generally low (Table 4.17) despite the sewage sludge soil containing a high soil metal concentration (Table 4.8).

Table 4.17 Tissue metal concentrations of plants grown in the sewage sludge soil. Mean plant tissue metal concentrations (μg/g) for: (a) flax, (b) miscanthus and (c) nettle. Values are the average of four replicate pots (n=4). Where the values are below the limit of detection these are indicated by L.D.

(a) Flax	Cd		Cr		Cu	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Roots	16.30	*3.58*	20.99	*6.95*	70.77	*18.39*
Stem + Leaf	2.62	*1.17*	L.D.		3.66	*1.88*
	Pb		Ni		Zn	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Roots	13.07	*8.72*	71.69	*17.20*	170.99	*42.26*
Stem + Leaf	L.D.		16.38	*7.36*	65.17	*29.49*

(b) Miscanthus	Cd		Cr		Cu	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Stem	17.64	*28.28*	L.D.		8.38	*4.40*
Leaf	43.08	*83.53*	1.77	*0.72*	11.49	*7.90*
	Pb		Ni		Zn	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Stem	L.D.		16.49	*11.47*	79.92	*57.74*
Leaf	L.D.		12.33	*18.87*	24.66	*13.44*

(c) Nettle	Cd		Cr		Cu	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Stem	0.36	*0.71*	L.D.		7.88	*0.49*
Leaf	L.D.		2.20	*4.40*	12.23	*2.90*
	Pb		Ni		Zn	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Stem	L.D.		29.31	*1.46*	79.46	*21.52*
Leaf	13.91	*4.99*	39.15	*3.85*	46.34	*16.94*

Flax sewage sludge

As observed in the control, T1 and T2 soils (Table 4.12, 4.13), flax plants grown in sewage sludge soil exhibited higher root tissue concentrations than shoot tissue concentrations for all of the metals studied (Table 4.17a). Both Cr and Pb were present in the root tissue of flax plants but were not present in detectable quantities in the above ground (stem + leaf) tissue (Table 4.17a). In the above ground tissue, Ni and Zn were present at the highest concentrations, 16 μg/g and 65 μg/g, respectively. In terms of the soil total metal concentration, the above ground tissue Ni was present at 4% and Zn at only 3%, whereas, in the root tissue these percentages were higher with, Ni and Zn present at 17% and 8%, respectively (Fig. 4.10). In the sewage sludge soil, Ni and Zn were the only metals in the above ground tissue present at higher concentrations than their corresponding concentrations in plants grown in the control soil (Table 4.12, 4.17).

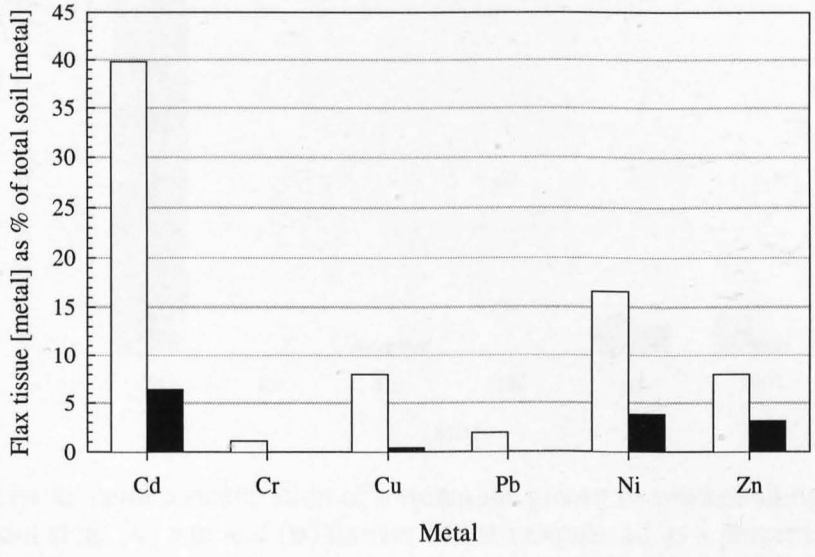

Figure 4.10 Tissue metal concentration of flax grown in sewage sludge soil. Values, expressed as a percentage of the soil total metal concentration, represent root (□) and stem + leaf (■) tissues.

Cadmium was present in above ground tissue at 6% of the soil total Cd concentration and as such was the most mobile metal in the flax–sewage sludge system, relative to the soil metal loading. The mobility of Cu and Pb in the Cu_{T1} and Pb_{T1} soils (the only surviving flax–T1 and flax–T2 soil treatments) was greater than the mobility of these metals in the sewage sludge soil (Fig. 4.7, 4.10).

Miscanthus sewage sludge

Cadmium was present in the stems of miscanthus grown in the sewage sludge soil at 18 μg/g, (Table 4.17b), a similar concentration to the miscanthus plants grown in the Cd_{T1} soil, however, the leaves of the plants grown in the Cd_{T1} contained four fold more Cd than the plants grown in the sewage sludge soil (Table 4.13, 4.17b). This high uptake of Cd from the sewage sludge soil by miscanthus highlighted Cd as the only metal more mobile in the sewage sludge soil than the corresponding T1 and T2 soils (Fig. 4.8, 4.11). However, uptake of Cd by miscanthus from the sewage sludge soil was variable; the result obtained was a mean of one replicate pot with a high cadmium concentration and another three replicate pots with lower cadmium concentrations.

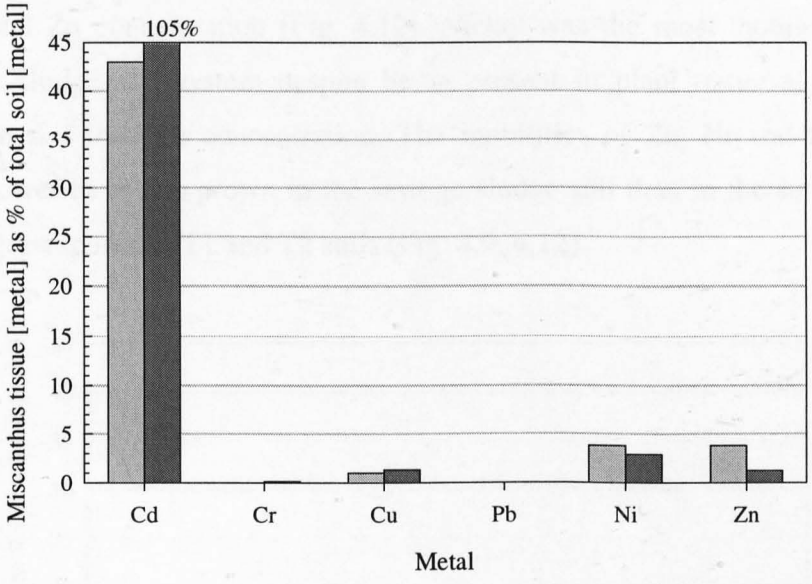

Figure 4.11 Tissue metal concentration of miscanthus grown in sewage sludge soil. Values represent stem (▒) and leaf (■) tissues and are expressed as a percentage of the soil total metal concentration. The data have been shown on a scale of up to 45%, for comparison with the flax and nettle (Fig. 4.10, 4.12), with the exception of cadmium leaf which has been labelled accordingly.

Zinc, unlike Cd, was present at a higher stem than leaf tissue metal concentration, than was observed for Zn_{T1}. Of all the metals Zn was taken up into miscanthus tissue at the highest concentration from the sewage sludge soil (80 μg/g in stems, Table 4.17b), however, this represented only 3.8% of the sewage sludge soil Zn concentration (Fig. 4.11). Although the sewage sludge soil had a total Zn concentration 16 fold greater than the control soil, miscanthus grown in the sewage sludge soil had Zn tissue concentrations lower than those of the control plants. Copper and Ni had low mobility in the sewage sludge soil–miscanthus system present at between 8 μg/g and 16 μg/g; this was < 1.4% and 4% of the sewage sludge soil total metal concentration for Cu and Ni, respectively. Lead was not

detectable in the miscanthus tissue and Cr was only detectable in miscanthus leaves thus these were the least mobile metals in the sewage sludge–miscanthus system.

With the exception of Cd, the mobility of the each of the metals in the sewage sludge soil was lower than the metal mobility in the corresponding T1 and T2 soils; this reduction in relative mobility was most evident when comparing Zn_{T1} soil and the sewage sludge soil (Fig. 4.8, 4.11).

Nettle sewage sludge

As with miscanthus plants, Zn was taken up by nettle stem tissue at the highest concentration (Table 4.17c), however, again this represented only 3.7% of the sewage sludge soil total Zn concentration (Fig. 4.12). Nickel was the most mobile metal in the nettle–sewage sludge soil system despite being present in plant tissue at <10% of the sewage sludge soil total Ni concentration. The mobilities of Zn, Ni and the remaining metals were lower in nettles grown in the sewage sludge soil than in the surviving nettles grown in the corresponding T1 and T2 soils (Fig. 4.9, 4.12).

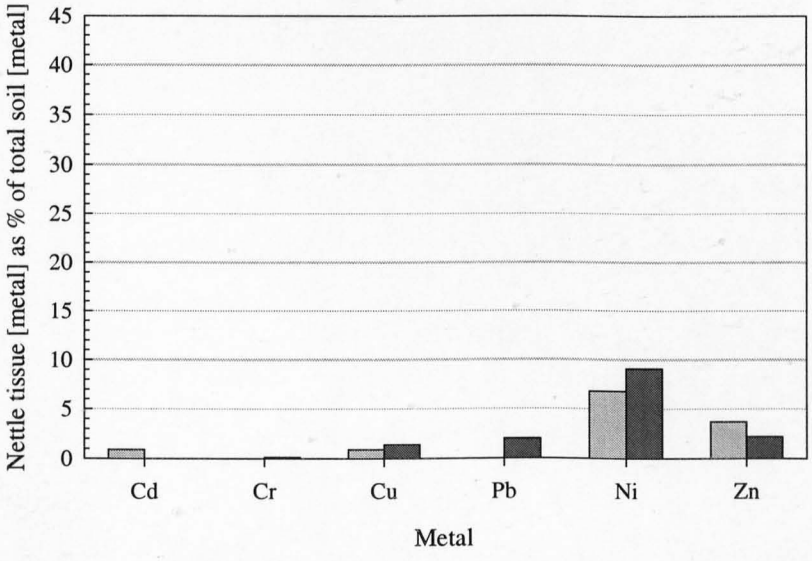

Figure 4.12 Tissue metal concentration of nettle grown in sewage sludge soil. Values represent stem (▒) and leaf (■) tissues and are expressed as a percentage of the soil total metal concentration.

Cadmium was found in tissues of both flax and miscanthus grown in sewage sludge soil at higher concentrations than in tissues of nettle, where it was only found in stem tissue (<1%). This result was surprising as the Cd_{T1}–nettle system had the highest metal mobility of all the metal–plant species systems in Pot Experiment One.

4.1.3.4 Metal uptake summary

In Pot Experiment One, the highest plant tissue metal concentration recorded was for Zn in the stems of miscanthus plants. At 969 μg/g the Zn concentration in miscanthus stems was approximately 100 μg/g higher than the concentration of Zn in miscanthus leaves. These results were of a similar order of magnitude to those obtained for Cd_{T1} in nettle stem and leaf tissues which had concentrations of 919 μg/g and 852 μg/g, respectively. These two combinations, the miscanthus–Zn_{T1} and nettle–Cd_{T1}, each with tissues concentrations between 800 and 1000 μg/g, gave above ground metal yields greater than twice that of any other plant–soil treatment combination in Pot Experiment One. The root tissue of flax plants grown in Pb_{T1} soil had the next highest metal concentration.

For each metal–plant species combination the metal mobility in the plant–sewage sludge soil system was lower than the corresponding plant–T1 or plant–T2 soil system (except Cd in miscanthus–sewage sludge soil), despite metal loadings of a similar order of magnitude. These metal mobilities indicated that the properties of the contaminated soils, such as soil organic matter content, are important factors in the possible success of any phytoremediation strategy.

4.2 Pot Experiment Two

Elucidating plant responses to marginally metal contaminated soils matrices

The aim of Pot Experiment Two was to study the survival of the plants and their metal uptake in soils containing the six metals in the study (Cd, Cr, Cu, Pb, Ni and Zn) at and closely exceeding the ICRCL threshold trigger values (Section 2.7; Table 2.8). Soils were made up to represent 100% and 120% of the ICRCL threshold trigger values (Section 2.7.1).

4.2.1 Soil metal concentrations

The metal concentrations of each of the Pot Experiment Two soils were derived from aqua regia digestion (Section 2.12.3.1) thus they represent the soil total metal concentrations (Tables 4.18–4.20).

4.2.1.1 Control soils

Three control soils were included in Pot Experiment Two (Table 4.18). The T3 and T4 soils were diluted in varying proportions in order to achieve the target concentration, thus two diluted control soils were produced to reflect the dilution range of the treated soils, control soils A and B (Section 2.7.1.1). Control soil C was unaltered soil from the Garscube walled garden which was a clay loam soil that had habitually received an annual application of farm yard manure prior to the study. Control soil C had low metal concentrations compared to the sewage sludge soil coupled with a high organic matter content and was therefore a suitable control for the sewage sludge soil.

Table 4.18 Pot Experiment Two control soil composition.

Control soil	*Composition*
A	30% of Pot Experiment One control
B	10% of Pot Experiment One control
C	Garscube soil

No Cd was detected in any of the control soils (Table 4.19a). As expected (Section 4.1.1.1) control soils A and B had low soil total metal concentrations and, with the exception of Cr, control soil A had higher soil total metal concentrations than control soil B. Of all the control soils, control soil C had the highest soil metal concentration for each of the metals considered. Apart from Cd, which was not detected, the soil metal concentrations in control soil C ranged from 25% to 80% of ICRCL threshold trigger values (Table 4.19b), representing an elevated metal loading.

Table 4.19 Pot Experiment Two control soil metal concentrations. Values below the limit of detection are indicated by L.D.

(a) Control soil total metal concentrations (μg/g). Standard deviations are shown (*st. dev.*); n = 3.

Control soil	Cd		Cr		Cu	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
A	L.D.		86.14	*19.18*	8.66	*0.50*
B	L.D.		107.64	*49.26*	4.11	*0.60*
C	L.D.		146.70	*42.34*	33.40	*2.35*

Control soil	Pb		Ni		Zn	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
A	77.73	*43.86*	39.40	*18.73*	44.73	*1.84*
B	18.87	*6.02*	25.86	*4.96*	23.30	*2.66*
C	153.13	*7.06*	55.82	*9.98*	170.55	*10.74*

(b) Control soil total metal concentrations expressed as a percentage of the ICRCL threshold trigger values

Control soil	Cd	Cr	Cu	Pb	Ni	Zn
	(%)	(%)	(%)	(%)	(%)	(%)
A	L.D.	14	7	16	56	15
B	L.D.	18	3	4	37	8
C	L.D.	24	26	31	80	57

4.2.1.2 T3 And T4 artificial soils

The values in Table 4.20 show the Pot Experiment Two T3 and T4 artificial soil total metal concentrations. The target concentrations, also shown, are 100% (T3) and 120% (T4) of the ICRCL threshold trigger values (Section 2.7; Table 2.8). The concentration achieved for T3 and T4 soils of Cd, Cr, Pb and Ni exceeded 100% and 120% of the ICRCL threshold trigger values, respectively; these soils were within 20% of the target concentration with the exception of the Cr_{T3}, Ni_{T3} and Ni_{T4} soils. The Zn and Cu T3 and T4 soil concentrations, however, fell below the 100% and 120% of the ICRCL threshold trigger values by up to 16% (Table 4.20).

Table 4.20 T3 and T4 target and actual soil total metal concentrations. Values presented are μg of metal extracted per g of dried, sieved and ground soils (Section 2.12.1.1). Standard deviations are shown (*st. dev.*); n = 3.

Treatment	Target concentration (μg/g)	Soil total metal concentration		
		(μg/g)	*st. dev.*	% of target concentration
Cd_{T3}	3	3.53	*0.58*	118
Cd_{T4}	4	4.64	*1.95*	116
Cr_{T3}	600	829	*40*	138
Cr_{T4}	720	846	*45*	118
Cu_{T3}	130	124	*10*	95
Cu_{T4}	156	131	*12*	84
Pb_{T3}	500	544	*32*	109
Pb_{T4}	600	619	*33*	103
Ni_{T3}	70	90	*13*	129
Ni_{T4}	84	103	*26*	123
Zn_{T3}	300	282	*14*	94
Zn_{T4}	360	330	*15*	92

4.2.1.3 Sewage sludge soil

The sewage sludge soil used for Pot Experiment Two was from the same source as used in Pot Experiment One (Section 2.6.1.4). The soil total metal concentrations for the sewage sludge soil (Table 4.21) were similar to those described in the Pot Experiment One (Tables 4.8 and 4.9). All the metals in the study were present at soil total metal concentrations in excess of the ICRCL threshold trigger values (Table 4.21).

Table 4.21 Sewage sludge soil total metal concentrations. Other details as table 4.20.

Treatment	Soil total metal concentration		
	(μg/g)	*st. dev.*	% of ICRCL threshold trigger values
Cd	44.25	*2.99*	1475
Cr	1856.53	*565.41*	309
Cu	902.82	*50.26*	694
Pb	756.43	*36.27*	151
Ni	489.69	*19.10*	700
Zn	1908.81	*185.06*	636

All the metal concentrations in the sewage sludge soil exceeded the ICRCL threshold trigger values by at least three fold with the exception of Pb which was 1.5 times the ICRCL threshold trigger value. With a low ICRCL threshold (3 μg/g) Cd was the most polluting metal in terms of the ICRCL guidelines. Copper, Ni and Zn were the next most contaminating metals in the sewage sludge soil. Considering the soil total metal concentrations alone, the sewage sludge soil appeared to be the most toxic soil in Pot Experiment Two by up to 11 fold more than the other soils.

4.2.2 Plant growth

4.2.2.1 Survival and height data

Height and survival data was recorded only for the flax and miscanthus plants (Fig. 4.13–4.16). Of the four plant species in the study, these two species grew tall with a measurable variation in height. Height data for the nettle and oilseed rape plants was not reported as these plants did not grow tall (<20 cm) thus it was not possible to accurately distinguish height variations between treatments. Analysis of the growth response of nettle and oilseed rape was based on yield data alone (Section 4.2.2.2).

4.2.2.1.1 Flax

Flax survival

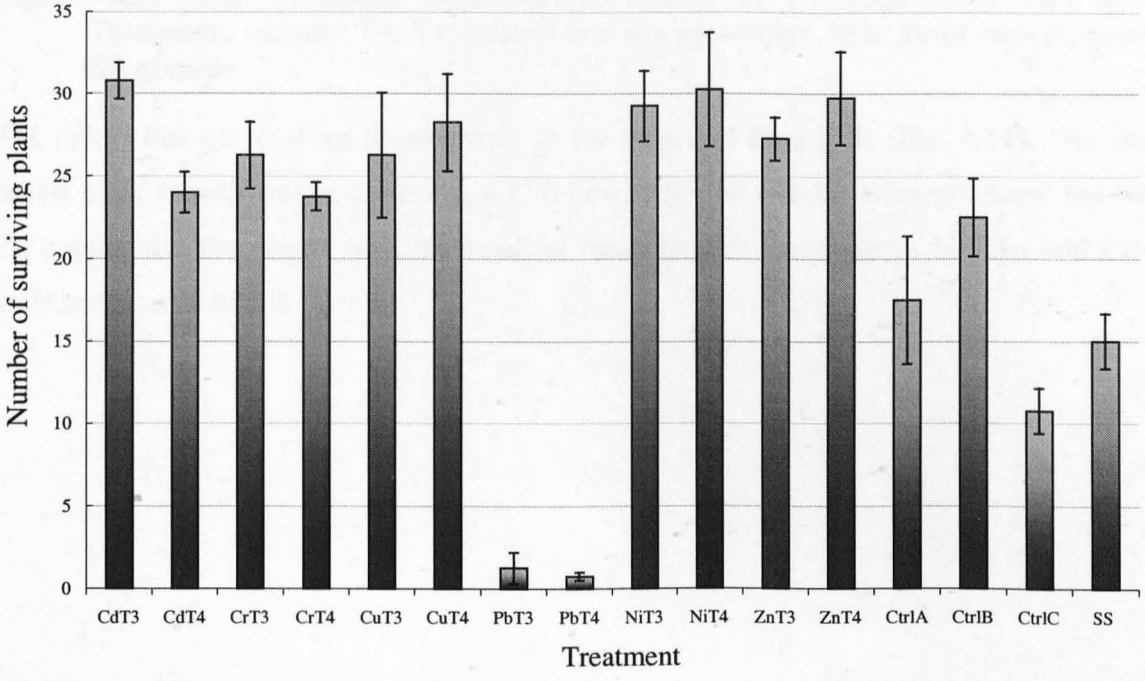

Figure 4.13 Mean survival of flax in Pot Experiment Two soils. Treatments include: T3, T4, control and sewage sludge soils. Error bars represent SE of mean.

Plants grown in the Cd_{T3} and Ni_{T4} soils had the best survival with a mean of over 30 plants per pot. For most of the treatments, a mean of 20–30 of the 50 flax seeds sown survived until the end of the growth period (Fig. 4.13). Plants grown in the control and sewage sludge soils gave lower mean survivals than the T3 and T4 soils with the exception of Pb in which survival was extremely poor. The poor survival of the plants grown in the Pb soils was due to a low emergence rate for these treatments, and was not consistent with either the germination study results or the Pot Experiment One results.

Flax height

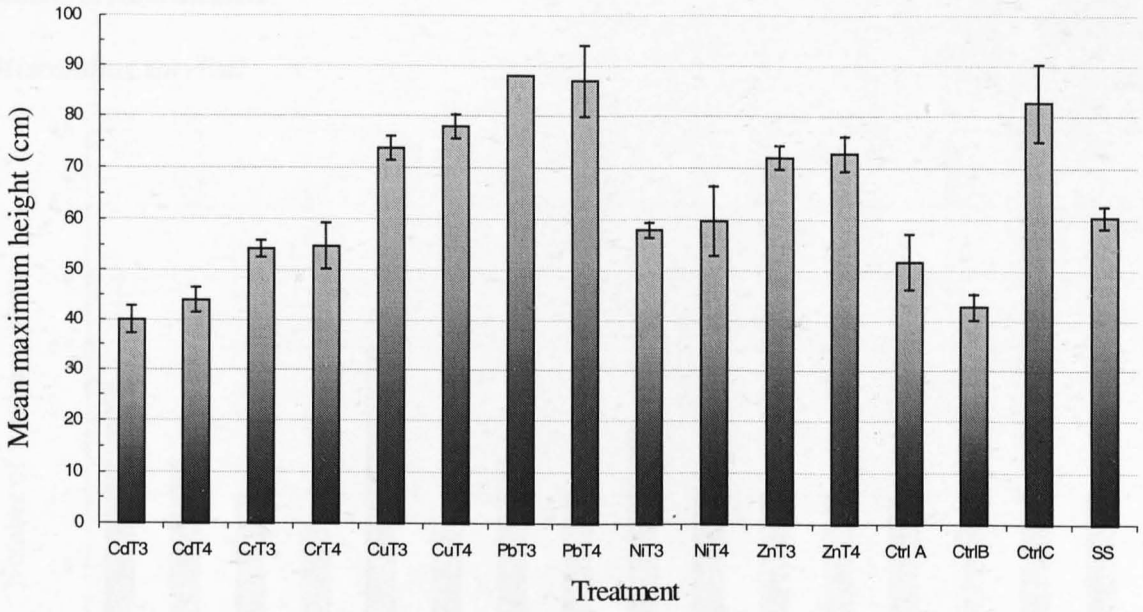

Figure 4.14 Mean maximum height of flax grown in Pot Experiment Two soils. Treatments include: T3, T4, control and sewage sludge soils. Error bars represent SE of mean.

The tallest flax plants were those grown in the Pb_{T3} and Pb_{T4} soils (Fig. 4.14). The next tallest plant growth was in control soil C followed by the two Cu treatments and the two Zn treatments. The plants with the smallest mean heights were grown in Cd_{T3} and Cd_{T4} soils and control soil B.

4.2.2.1.2 Miscanthus

Miscanthus survival

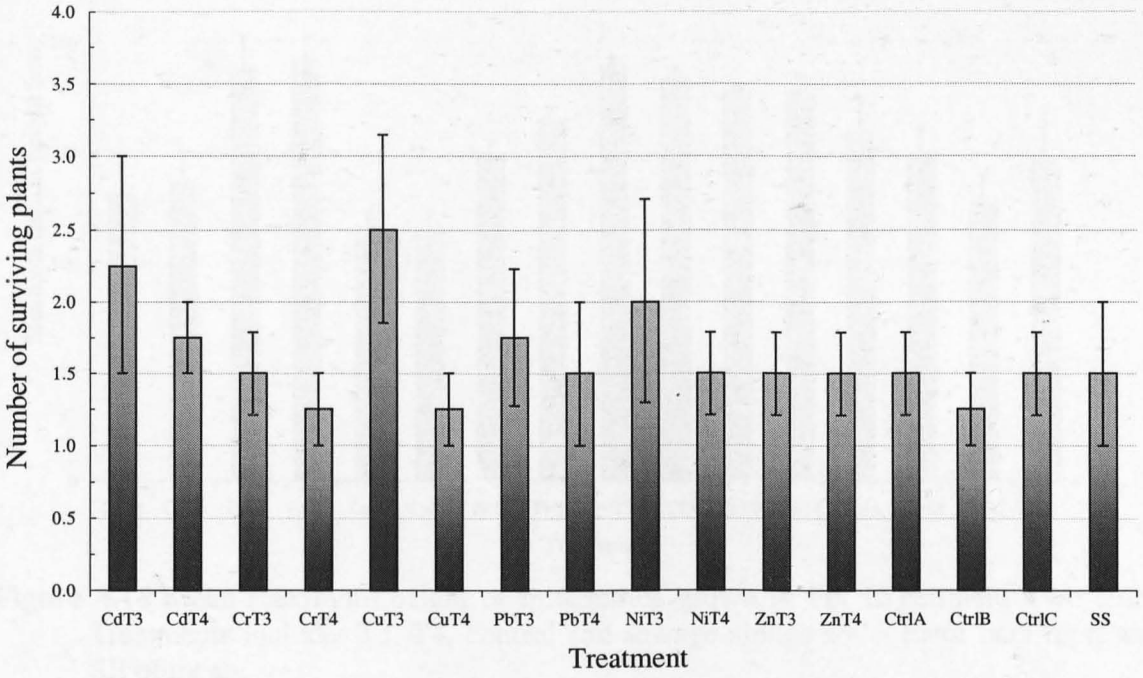

Figure 4.15 Mean survival of miscanthus in Pot Experiment Two soils. Treatments include: T3, T4, control and sewage sludge soils. Error bars represent SE of mean.

Miscanthus, grown form rhizome pieces, gave a lower and more uniform survival than flax (Fig. 4.15). Most of the treatments had a mean of 1–2 plants surviving per pot at the end of the growth period. Differences in the number of plants surviving in each pot were likely due to the number of active buds on the rhizome pieces and therefore did not necessarily reflect effect of the metals on miscanthus.

Miscanthus height

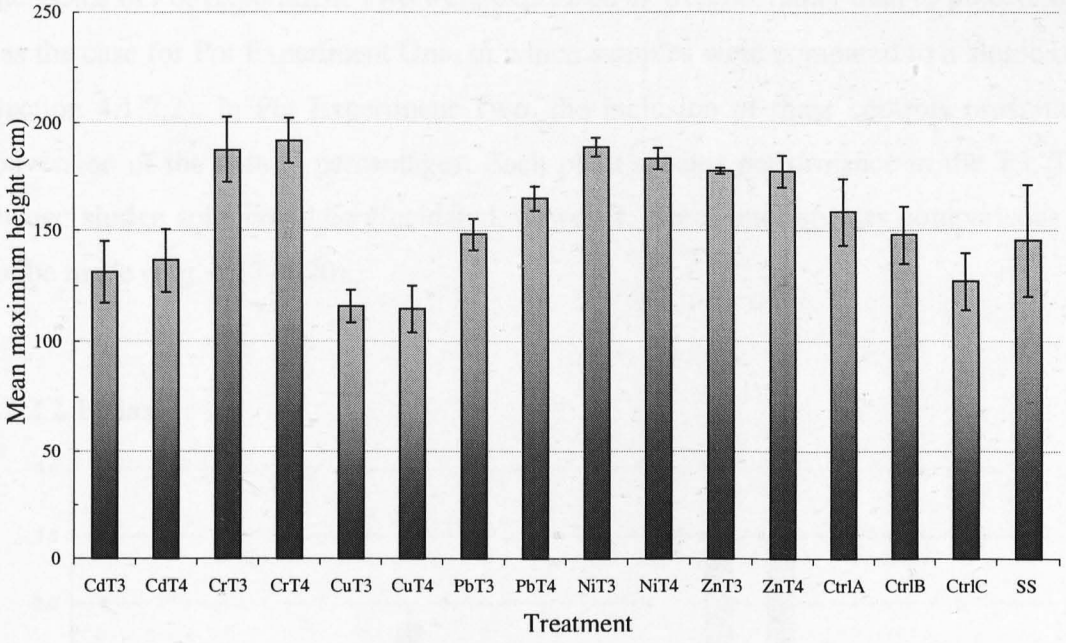

Figure 4.16 Mean maximum height of miscanthus grown in Pot Experiment Two soils. Treatments include: T3, T4, control and sewage sludge soils. Error bars represent SE of mean.

The tallest miscanthus plants were those grown in the Cr, Ni and Zn artificial soils and had similar heights at 177–191 cm (Fig. 4.16), close to heights of 2 m reported by Bullard and Kilpatrick (1997). For these three metals there was little difference between the height response of the plants grown in the T3 and T4 soils. The next tallest plants were Pb_{T4} and control A. The poorest heights were found in control soil C and the Cu soils.

4.2.2.2 Yield data

The yields in Pot Experiment Two were expressed as weights rather than as percentages, as was the case for Pot Experiment One, in which samples were compared to a single control (Section 4.1.2.2). In Pot Experiment Two, the inclusion of three controls prevented the conversion of the data to percentages. Each plant species performance in the T3, T4 and sewage sludge soils could be elucidated, however, direct inter-species comparisons could not be made (Fig. 4.17–4.20).

4.2.2.2.1 Flax

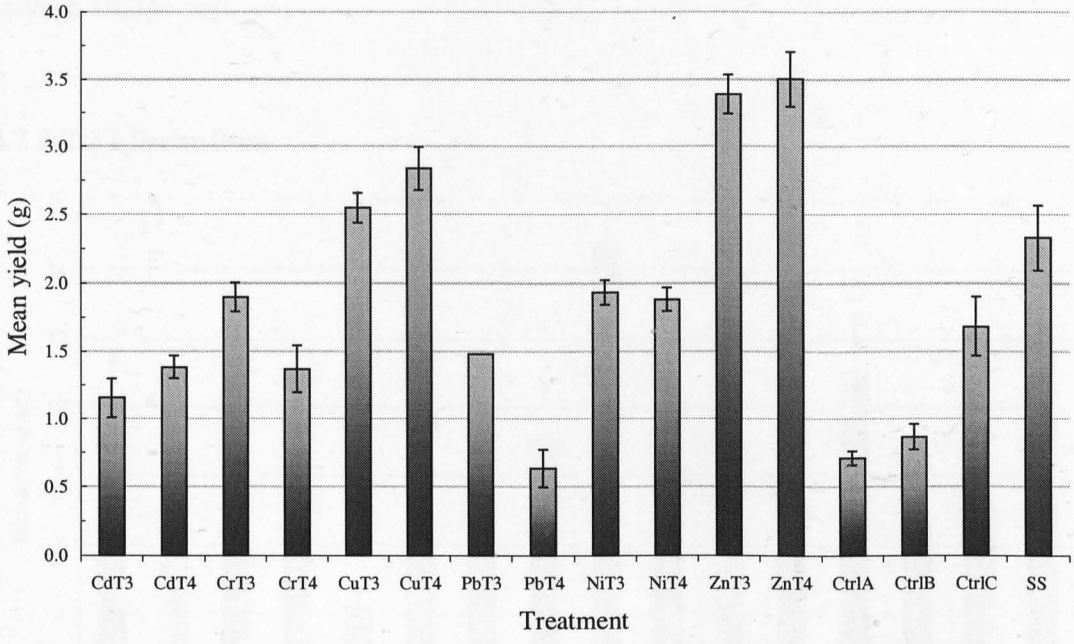

Figure 4.17 Air dried yields of flax tissue from plants grown in T3 and T4 soils. Yields of above ground plant tissue in each Pot Experiment Two treatment are expressed as a mean of the air dried biomass per pot. Error bars represent SE of mean.

Flax plants yielded above ground biomass in the range 0.6–3.5 g (Fig. 4.17). The flax plants grown in the control soils did not produce high yields compared with flax plants grown in the other treatments in the study. Control soils A and B yields were among the poorest of all the flax yields (0.7 g and 0.9 g respectively). The lowest yield for flax in Pot Experiment Two was from plants grown in the Pb_{T4} soil; both Pb treatments gave low yields, despite producing tall plants (Section 4.2.2.1.1), due to the low number of plants surviving until the end of the growth period. The Zn soils gave the highest yields with Zn_{T4} yielding slightly more than Zn_{T3}, at 3.5 g and 3.4 g respectively.

Cadmium, Cu and Zn had slightly higher yields in the T4 soils than in the T3 soils (Fig. 4.17) indicating that the increase in soil total metal concentration did not inhibit plant biomass production. The difference in yields between the T3 and T4 soils was not such that

the T4 treatments could be considered to have had any promotional effect on flax biomass production. The yields of the Ni_{T3} and Ni_{T4} treatments were very similar, whilst the remaining two metals, Cr and Pb, showed a marked decrease in yield from the T3 to the T4 soils. These data indicated that in the region of the ICRCL threshold trigger values, Cr and Pb were the only metals to exhibit an inhibitory effect on flax biomass production in response to the increase in soil total metal concentration.

Flax biomass production in the sewage sludge soil was greater than the three control treatments (Fig. 4.17). The sewage sludge soil also yielded greater flax biomass than each of the Cd, Cr, Pb and Ni soils. Only the Cu and Zn soils produced a higher yield than the sewage sludge soil.

4.2.2.2.2 Miscanthus

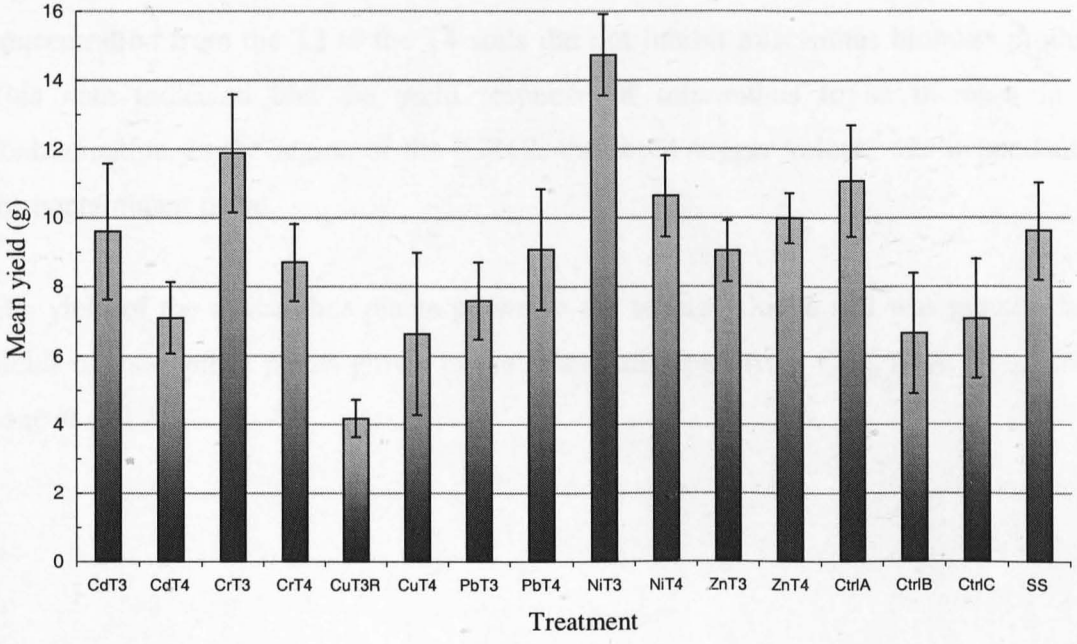

Figure 4.18 Air dried yields of miscanthus tissue from plants grown in T3 and T4 soils. Yields of above ground plant tissue in each Pot Experiment Two treatment are expressed as a mean of the air dried biomass per pot. The Cu_{T3} treatment is labelled "CuT3R" where "R" denotes the yield as a mean taken from replanted pots which had a five week growth disadvantage (Section 2.7.2). Error bars represent SE of mean.

Miscanthus was the highest yielding of all the plants grown in Pot Experiment Two with above ground biomass mean yields ranging from 4.2 g (Cu) to 14.7 g (Ni) (Fig. 4.18). The control treatments did not produce the greatest yields, however, the miscanthus plants growing in the control soils performed better than the flax plants growing in the control soils, especially with respect to control soil A. Several of the miscanthus pots failed to produce shoots, which was likely attributable to the rhizome pieces having no viable

nodes. Where shoots failed to appear, the pots were replanted with new rhizome pieces, however, these pots were at a five week disadvantage over the growth period compared to the other treatments (Section 2.7.2). If a treatment had some pots which failed and others which were successful then only the successful treatments were used to calculate the mean yield. The Cu_{T3} soil was the only treatment in which shoots failed to appear in all replicate pots, this was reflected in the low yield observed (Fig. 4.18).

The highest yielding treatment was Ni_{T3} (14.7 g) whilst the lowest yielding treatment was the replanted Cu treatment Cu_{T3} (4.2 g) (Fig. 4.2). For three of the six metals, Cd, Cr, and Ni, the lower soil total metal concentration (T3) gave a higher yield than the higher soil total metal concentrations (T4), suggesting the metals were inhibiting plant growth. The metal which produced the greatest reduction in yield from the T3 to the T4 soil was Ni with a reduction of 28%. The remaining two metals, Pb and Zn, gave higher yields in the higher than the lower soil total metal concentrations, indicating the increase in soil metal concentration from the T3 to the T4 soils did not inhibit miscanthus biomass production. This data indicated that the yield response of miscanthus to an increase in metal contamination, in the region of the ICRCL threshold trigger values, was dependant upon the contaminant metal.

The yield of the miscanthus plants grown in the sewage sludge soil was greater than the yields of miscanthus plants grown in the other soils apart from Cr_{T3}, Ni_{T3}, Ni_{T4}, Zn_{T4} and control soil A.

4.2.2.2.3 Nettle

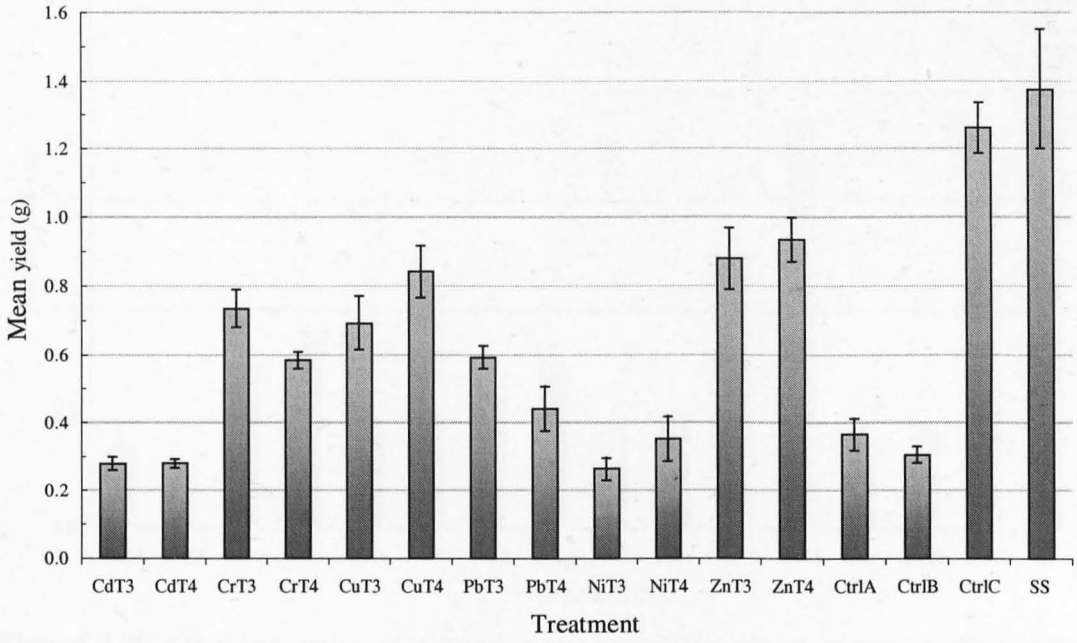

Figure 4.19 Air dried yields of nettle tissue from plants grown in T3 and T4 soils. Yields of above ground plant tissue in each Pot Experiment Two treatment are expressed as a mean of the air dried biomass per pot. Error bars represent SE of mean.

The yields of the nettle plants grown in the Pot Experiment Two soils were low compared to the yields of flax and miscanthus (Fig. 4.17–4.19). The nettle plants grown in the sewage sludge soil produced the highest biomass of all the treatments followed by control soil C (Fig. 4.19). These two soils were the most fertile of the Pot Experiment Two soils and the nettle plants responded well to the soil properties of these treatments relative to the other treatments. In contrast, the two artificial control soils, control soils A and B, were among the six poorest yielding soils in the experiment (0.4 and 0.3 g of above ground biomass, respectively). The poorest yields were produced in the two Cd soils, both yielding 0.28 g and the Ni_{T3} and Ni_{T4} soils (0.26 g and 0.35 g, respectively). $Zinc_{T4}$ and Zn_{T3}, produced the highest yielding nettle plants grown in the T3 and T4 soils followed by the Cu_{T4}, Cr_{T3} and Cu_{T3} soils. Chromium and Pb produced lower yields in the T4 than in the T3 soil treatments indicating increased inhibition of growth as the metal concentrations increased.

4.2.2.2.4 Oilseed Rape

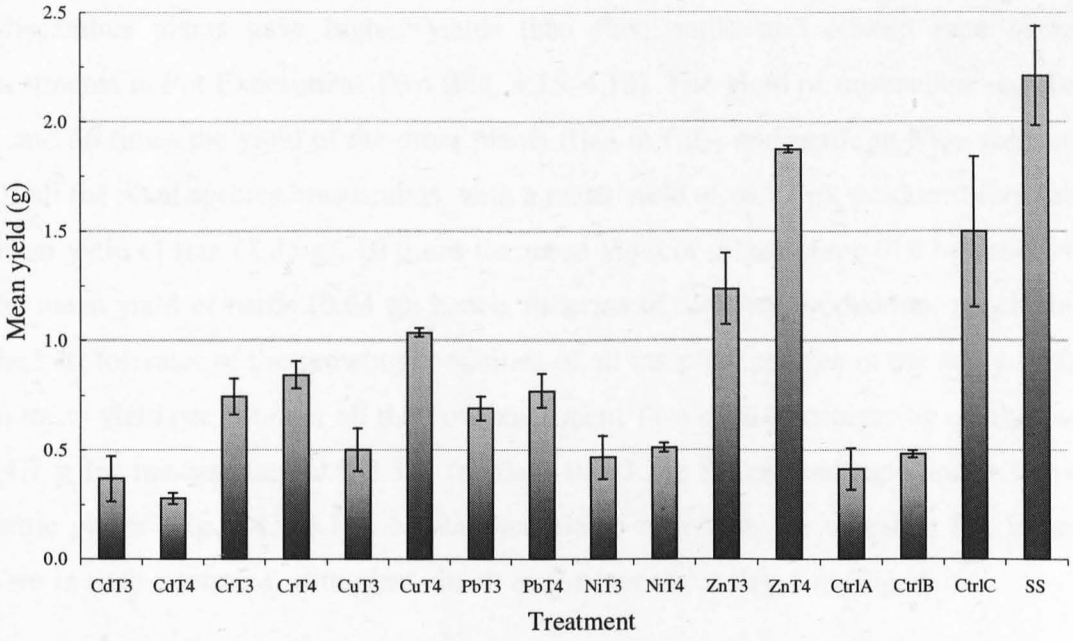

Figure 4.20 Air dried yields of oilseed rape tissue from plants grown in T3 and T4 soils. Yields of above ground plant tissue in each Pot Experiment Two treatment expressed as a mean of the air dried biomass per pot. Error bars represent SE of mean.

Like the nettle plants the yields of the oilseed rape plants were low and again the sewage sludge soil produced the highest yield at 2.2 g (Fig. 4.20). An infestation of cabbage white caterpillars resulted in a significant loss of plant tissue over the growth phase of the experiment and contributed to the low yields recorded for the oilseed rape plants. $Zinc_{T4}$ was the second highest yielding treatment at 1.9 g followed by control soil C (1.5 g). The two artificially made control soils controls A and B produced among the lowest yielding plants in Pot Experiment Two. The only metal in which the T4 soil gave a lower yield than the T3 soil was Cd, which also gave the poorest yields (0.4 and 0.3 g, respectively). The other five metals gave higher yields in the T4 than the T3 soils, however, only Cu and Zn gave a large difference in yield between the T3 and T4 treatments (Fig. 4.20). For these two metals the yields were not adversely affected by increased soil metal loading as the higher concentrations resulted in a yield gain.

4.2.2.3 Growth summary

Miscanthus plants gave higher yields than flax, nettle and oilseed rape for all the treatments in Pot Experiment Two (Fig. 4.15–4.18). The yield of miscanthus was between 2 and 56 times the yield of the other plants (flax in Cu_{T3} and nettle in Ni_{T3}, respectively). Of all the plant species, miscanthus, with a mean yield of (8.97 g), produced five times the mean yield of flax (1.85 g), 10 times the mean yield of oilseed rape (0.87 g) and 14 times the mean yield of nettle (0.64 g); hence, in terms of biomass production, miscanthus was the best tolerator of the growing conditions of all the plant species in the study. The range in mean yield per pot over all the Pot Experiment Two soils in decreasing order, was: 1.9–14.7 g for miscanthus , 0.6–3.5 g for flax, 0.3–2.2 g for oilseed rape and 0.3–1.4 g for nettle plants (Fig. 4.15–4.18). Miscanthus plants were also the tallest in Pot Experiment Two in each treatment, with plants in all treatments exceeding 1 m (Fig. 4.16).

It was not surprising that miscanthus should be established as having the best growth response to the conditions in Pot Experiment Two as the plant species is a massive perennial grass and was grown in relatively hot conditions which were favourable to miscanthus plants. The growth of the other plants in the study, particularly the oilseed rape and the nettles, was poor and again this was probably in part due to the hot growing conditions which were unfavourable to these temperate broad leafed plants.

Another likely contributory factor to the poor growth of these plants was the poor nutrient status of all the treated soils in the study. The inclusion of control soil C and the sewage sludge soil tested the plants response to nutrient status and soil physical properties; indeed, both oilseed rape and nettle gave higher yields in the sewage sludge soil and control C soils in than all but one of the other soils, confirming their preference for soils with a higher nutrient status. Notwithstanding, the yields of these treatments were disappointing. Flax yields were greater than oilseed rape and nettle therefore flax showed greater potential as a tolerator of the unfavourable growing medium.

The highest yielding plant-treatment combination was the miscanthus plant-Ni_{T3} soil system. In striking contrast, the same soil also produced the lowest yielding plant-treatment combination with nettle plants. This gave a clear demonstration that it is not possible to generalise how plants will respond to contaminated soils. The yield response of the miscanthus plants to the Ni_{T3} soil was in fact not typical of the plant species in the study as for flax, nettle and oilseed rape the Zn and Cu soil treatments all gave better yields than the Ni soils (Fig. 4.15–4.18).

For all the plants species in the study, the growth response of the plants to control soils A and B was poor in comparison to the other soil treatments. This indicated that metals at around the ICRCL threshold trigger values did not inhibit plant growth in the contaminated matrix used in the study.

Plants of all four species grown in the sewage sludge soil gave favourable yield responses compared to the T3, T4 and control soils; yields of flax and miscanthus grown in the sewage sludge soil were within the top six yielding soil treatments for these plant species, whilst nettle and oilseed rape grown in the sewage sludge soil had highest yields of all treatments for these plant species. The sewage sludge soil gave these high yields in spite of the high loading of multiple contaminant metals. The low availability of the metals and the high nutrient status of the sewage sludge soil contributed to the high yields of the plants grown in the soil. The growth response of the nettle and oilseed rape plants to the nutrient rich and well structured control C and sewage sludge soils suggested that significant improvements in the yields of these plants could be made by providing nutrients as part of a phytoremediation programme.

4.2.3 Plant tissue metal concentrations

Miscanthus was established as the plant species producing the greatest biomass (Section 4.2.2.2), however, to be a suitable candidate for phytoremediation, a plant species must also have the ability to transport metal from the rhizosphere to above ground plant tissues. Measurement of the metal concentration within the plant tissue can give an insight into the species' ability to accumulate metal. The plant tissue metal concentration, when expressed as a percentage of the soil metal concentration (Fig. 4.21–4.26), showed the plants' ability to take up metal, with respect to the soil metal loading, and allowed direct comparison between the mobility's of the six metals. Plant tissue metal concentrations > 100% of the soil metal concentration represented an accumulation of the metal. In Pot Experiment One (Section 4.1.3), some of the stem and leaf tissues were analysed separately, however, in Pot Experiment Two, all above ground biomass was consistently analysed as pooled stem and leaf tissue, which allowed clear inter-plant species comparisons in aerial plant tissue metal concentrations to be made. Metal uptake of each of the six metals was considered separately for the four plant species under investigation before comparisons between all of the metal–plant combinations were made.

4.2.3.1 Control soils

Studying the uptake of the metals to above ground tissues in the plant species–control soil systems allowed the behaviour of the metals at background levels in soils, with differing organic matter contents, to be elucidated. Soil total metal concentrations for the control soils are detailed in Section 4.2.1.1.

Cadmium

The control soils, despite having soil Cd concentrations below the limit of detection (Table 4.19), produced plant tissue Cd concentrations of up to 2.58 μg/g. Oilseed rape was the plant species able to accumulate the greatest tissue concentration of Cd closely followed by flax (Table 4.22). Nettle growing in control soil A was the only plant–control soil system which had a Cd tissue concentrations below the limit of detection.

Table 4.22 Tissue metal concentrations of plants grown in the control soils. Mean plant tissue metal concentrations (μg/g) of Cd, Cr, Cu, Pb, Ni, and Zn in: flax, miscanthus, nettle and oilseed rape. Values are calculated from four replicate pots (n=4). L.D. denotes plant tissue metal concentration below the limit of detection.

	Control soil	Flax		Miscanthus		Nettle		Oilseed rape	
Cd		(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
	A	2.32	*0.79*	0.58	*0.67*	L.D.		2.57	*0.63*
	B	2.47	*0.33*	1.04	*0.71*	1.47	*0.87*	2.58	*0.91*
	C	2.41	*0.50*	1.75	*0.74*	2.41	*0.50*	2.24	*0.68*

	Control soil	Flax		Miscanthus		Nettle		Oilseed rape	
Cr		(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
	A	L.D.		L.D.		L.D.		L.D.	
	B	7.48	*n=1*	L.D.		L.D.		L.D.	
	C	L.D.		L.D.		L.D.		L.D.	

	Control soil	Flax		Miscanthus		Nettle		Oilseed rape	
Cu		(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
	A	1.00	*1.22*	L.D.		13.94	*7.91*	0.79	*n=1*
	B	1.63	*0.75*	0.25	*n=1*	10.16	*3.76*	3.29	*3.1*
	C	1.58	*1.83*	L.D.		3.16	*0.24*	0.37	*n=1*

	Control soil	Flax		Miscanthus		Nettle		Oilseed rape	
Pb		(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
	A	L.D.		L.D.		L.D.		L.D.	
	B	L.D.		L.D.		L.D.		L.D.	
	C	L.D.		L.D.		L.D.		L.D.	

	Control soil	Flax		Miscanthus		Nettle		Oilseed rape	
Ni		(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
	A	5.74	*1.87*	7.41	*3.30*	4.70	*3.54*	24.41	*24.16*
	B	8.85	*3.21*	12.57	*8.28*	5.13	*3.73*	7.62	*3.53*
	C	7.64	*2.44*	27.74	*33.11*	7.64	*2.44*	5.31	*1.03*

	Control soil	Flax		Miscanthus		Nettle		Oilseed rape	
Zn		(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
	A	40.39	*2.92*	38.19	*6.53*	48.02	*15.58*	64.21	*32.73*
	B	26.49	*3.45*	28.73	*3.31*	35.50	*13.95*	37.82	*8.72*
	C	28.17	*5.03*	22.73	*2.61*	28.17	*5.03*	28.36	*2.93*

Chromium

All the plant species grown in the control soils had tissue Cr concentrations below the limit of detection, with the exception of flax plants grown in control soil B (Table 4.22). The behaviour of Cr in the control soils directly contrasted with Cd; what little Cd was present in the control soils was taken up by the plant species, however, Cr was not detectable in the majority of plants growing in the control soils despite being present in the soils at 14–24% of its ICRCL threshold trigger value (Table 4.19). The one plant–soil system which produced a detectable Cr tissue concentration, flax–control soil B, gave a low plant tissue concentration of 7% of the soil total Cr concentration (Table 4.23).

Table 4.23 Uptake and accumulation of metals by plants grown in the control soils. Values shown are the mean control soil plant tissue metal concentrations expressed as a percentage of the soil total metal concentrations for Cr, Cu, Ni, and Zn. Mean tissue Pb concentrations could not be calculated as the plant tissue concentrations were below the detection limit and have been omitted: tissue concentration below the detection limit for other metals are denoted by "-".Where plant tissue concentration are > 100% the figures are highlighted in bold. Tissue concentrations above the detection limit for Cd, where the soil metal concentration was less than the detection limit are denoted by "+".

Flax	Control soil	Cd	Cr	Cu	Ni	Zn
	A	+	-	12	15	90
	B	+	7	40	34	**114**
	C	+	-	5	14	17
Miscanthus	Control soil	Cd	Cr	Cu	Ni	Zn
	A	+	-	-	19	85
	B	+	-	6	49	**123**
	C	+	-	-	50	13
Nettle	Control soil	Cd	Cr	Cu	Ni	Zn
	A	-	-	**161**	12	**107**
	B	+	-	**147**	20	**152**
	C	+	-	9	14	17
Oilseed rape	Control soil	Cd	Cr	Cu	Ni	Zn
	A	+	-	9	62	**144**
	B	+	-	38	29	**162**
	C	+	-	1	10	17

Copper

The maximum tissue Cu concentrations for miscanthus, oilseed rape and flax were all found in the plants grown in control soil B, whilst in nettles the maximum tissue Cu concentration was found in the plants grown in control soil A (Table 4.22). The higher uptake from control soils A and B than from control soil C did not correlate with the soils Cu loading as control soil C had four times and eight times more Cu than control soils A and B, respectively. Thus the control soils with a lower organic matter, control soils A and B, had a higher Cu uptake than control soil C despite its' higher Cu loading. Nettle plants grown in control soil A had the highest tissue Cu concentration of all the plant species–control soil systems with a tissue concentration ~ four fold that of the plant species with the next highest tissue Cu concentration, oilseed rape grown in control soil B.

Copper was one of two metals most accumulated from the control soils, as the nettle tissue Cu concentration in plants grown in control soil A was 161% of the control soil A total Cu concentration (Table 4.23).

Lead

Lead was present in control soil A at approximately one sixth of the ICRCL threshold trigger value and in control soil C at one third of the ICRCL threshold trigger value (Table 4.19), despite this significant soil loading of Pb, none of the plants growing in the control soils had any detectable Pb in their tissues (Table 4.22).

Nickel

Control soil C had the highest soil total Ni concentration of all the Pot Experiment Two controls at 56 μg/g (Table 4.19). Miscanthus–nickel and nettle–nickel were the only two of the 24 plant–metal systems where the plant tissue metal concentrations were greater in the plants grown in control soil C than in control soils A and B (Table 4.22). Nickel was taken up in the greatest quantity by miscanthus grown in control soil C, however, due to the high soil metal loading of control soil C, Ni was most mobile in the oilseed rape–control soil A system (Table 4.23).

Zinc

In control soils A and B, Zn was present at 15% and 7.6% of the ICRCL threshold trigger value, respectively, but Zn was more concentrated in control soil C at 57% of the ICRCL threshold trigger value (Table 4.19). Despite the higher Zn loading in control soil C, Zn uptake was highest, for all the plant species, from control soil A (Table 4.22). Plants from the oilseed rape–control soil A system had the highest tissue Zn concentration. All the plant species grown in control soils A and B had a Zn uptake of $\geq$ 85% of the soil total Zn concentration whereas all the plant species grown in control soil C had a Zn uptake of < 18% of the soil total Zn concentration (Table 4.23). Zinc uptake in controls soils A and B was consistently high for all of the plant species in the study; out of the eight soil–plant species systems, six accumulated a Zn tissue concentration in excess of the soil Zn concentration. Conversely, uptake of Zn was consistently low from control soil C across all the plant species in the study (Table 4.23). Zinc was the metal present in each plant species' tissue at the highest concentration with the exception of Ni in the miscanthus–control soil C system and was the most mobile metal in all but two plant species–soil systems, the exceptions being Ni in the miscanthus–control soil C system and Cu in the nettle–control soil A system.

4.2.3.2 T3 And T4 artificial soils

It was of key interest to elucidate above ground tissue metal concentrations of the plant species grown in the T3 and T4 soils in order to identify suitable phytoremediators for the six metals in the study. The soil total metal concentrations for the T3 and T4 soils were detailed in Section 4.2.1.2.

Table 4.24 Tissue metal concentrations of plant species grown in the T3 and T4 soils. Mean plant tissue metal concentrations (μg/g) for flax, miscanthus, nettle and oilseed rape. Unless otherwise indicated there were 4 replicate samples (n=4), † denotes n=3 and ✳ denotes a single sample with 3 analytical replicates. This result (✳) did not take part in the *t* test. For each element values sharing the same letter are not significantly different (*t* test using Bonferroni correction, $P < 0.05$).

	Mean stem and leaf metal concentration								
	Cd			Cr			Cu		
	(μg/g)	*st. dev.*	*t* test	(μg/g)	*st. dev.*	*t* test	(μg/g)	*st. dev.*	*t* test
Flax T3	38.16	*6.20*	ab	18.10	*6.50*	ab	2.83	*1.34*	a
Flax T4	48.77	*3.43*	a	23.27	*5.84*	ab	6.16	*1.66*	a
Miscanthus T3	2.41	*2.81*	c	8.67	*4.99*	a	8.90	*2.46*	a
Miscanthus T4	3.66	*2.53*	c	2.80	*0.32*	a	7.40	*6.71*	a
Nettle T3	12.00	*14.73*	bcd	11.72	*8.16*	ab	28.63	*3.75*	b
Nettle T4	10.34	*5.16*	bcd	15.62	*4.22*	ab	35.34	*6.19*	bc
Oilseed rape T3	38.77	*17.14*	ad	21.34	*15.42*	ab	49.73	*10.67*	c
Oilseed rape T4	52.21	*22.68*	a	34.71	*18.23*	b	38.37	*7.62*	bc
	Pb			Ni			Zn		
	(μg/g)	*st. dev.*	*t* test	(μg/g)	*st. dev.*	*t* test	(μg/g)	*st. dev.*	*t* test
Flax T3	23.3	*5.73*✳	✳	42.00	*3.70*	ab	275	*4*	a
Flax T4	32.0	*14.46*†	ab	56.91	*5.37*	ab	321	*27*	a
Miscanthus T3	75.7	*14.20*	ab	9.86	*2.18*	a	224	*54*	a
Miscanthus T4	61.6	*30.08*	ab	15.89	*3.23*	a	261	*44*†	a
Nettle T3	107.3	*37.32*†	a	90.78	*35.35*	b	1385	*241*	b
Nettle T4	87.8	*32.42*	ab	189.68	*56.11*	c	1937	*154*	c
Oilseed rape T3	22.7	*6.72*†	b	166.26	*45.76*	c	707	*91*	d
Oilseed rape T4	62.8	*22.80*	ab	187.14	*20.01*	c	995	*146*	d

Cadmium

An increase in plant tissue Cd concentration from the T3 to the T4 soils (Table 4.24) was observed for miscanthus, flax and oilseed rape. Nettle had a small reduction (<2 μg/g) in plant tissue Cd concentrations from the T3 to T4 soil. However, within each plant species, none of the differences between T3 and T4, for each metal, were significant (Table 4.24).

The highest plant uptake of Cd was found in flax and oilseed rape, with the T4 soils producing flax and oilseed rape plant tissue concentrations of 48.77 μg/g and 52.21 μg/g, respectively (Table 4.24); these tissue concentrations represented a significant accumulation of Cd from the soil matrix (Fig. 4.21). In both T3 and T4 soils, flax and oilseed rape had tissue Cd concentrations between 10 and 12 fold the soil Cd concentration; these were the only soil–plant species systems in Pot Experiment Two where an accumulation of >1000% of the soil metal concentration was observed in the plant tissue (Table 4.24 and Fig. 4.21–4.26). Nettle plants also accumulated Cd from both T3 and T4 soils, the greater accumulation being in the T3 soil where the tissue concentration was 340% of the soil concentration (Fig. 4.21). Cadmium, in the oilseed rape– and flax–T3 and T4 soil systems, however, was the most highly mobile metal observed in the Pot Experiment Two.

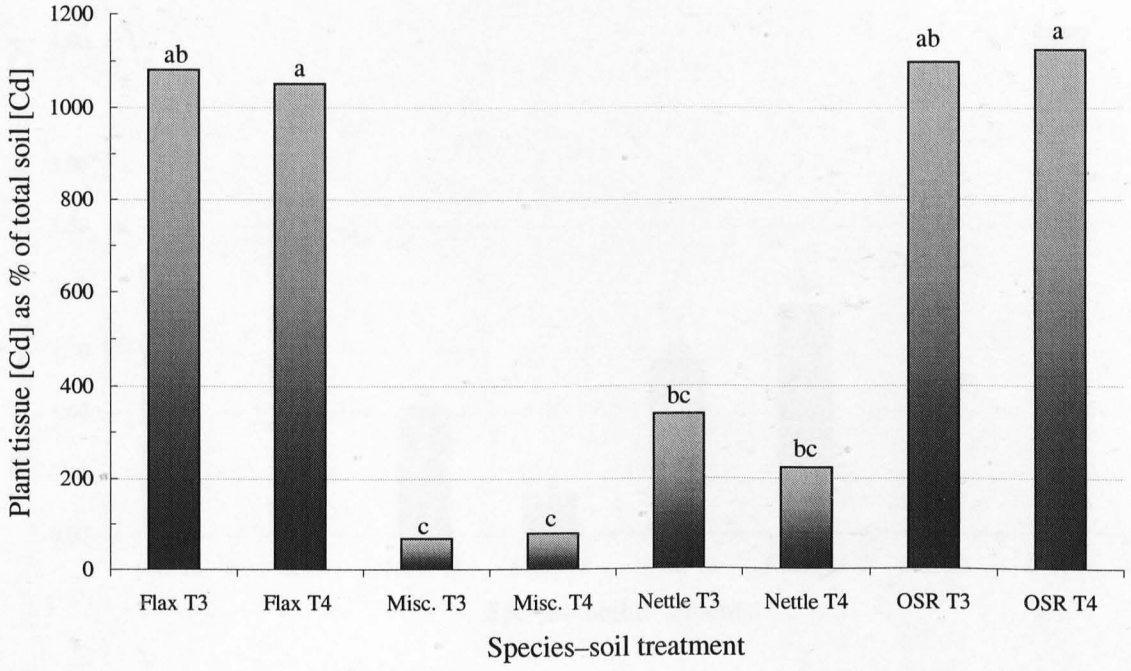

Figure 4.21 Stem and leaf tissue Cd concentrations of plant species grown in T3 and T4 soils. Plant tissue Cd concentration expressed as a % of soil total Cd concentration (Table 4.20, 4.24) for flax, miscanthus (Misc.), nettle and oilseed rape (OSR). Bars sharing the same letter are not significantly different (*t* test using Bonferroni correction, $P < 0.05$).

Only miscanthus plants failed to accumulate Cd, with tissue concentrations in the T3 and T4 soils of 68% and 79% of the soil concentration, respectively. The Cd concentration in miscanthus tissue grown in the Cd_{T3} soil was the lowest tissue concentration observed in all of the T3 and T4–plant species systems.

Chromium

Uptake of Cr from the soil matrix was consistently low for all the plant species studied and only the oilseed rape–T4 soil and the miscanthus–T3 and T4 soils were significantly different (Fig. 4.22). Miscanthus had the lowest Cr uptake with a mean tissue concentration of 2.80 μg/g for the plants grown in the Cr_{T4} soil, which was only 0.33% of the soil Cr loading. Miscanthus plants had lower tissue Cr concentrations in the plants grown in the Cr_{T4} soil than the Cr_{T3} soil (Table 4.24). Flax, oilseed rape and nettle plants grown in the Cr_{T4} soils all contained higher tissue Cr concentrations than the plants grown in the T3 soils. Of all the T3– and T4–plant species systems in Pot Experiment Two, the miscanthus–Cr_{T4} soil system gave the lowest uptake with respect to the soil total metal loading.

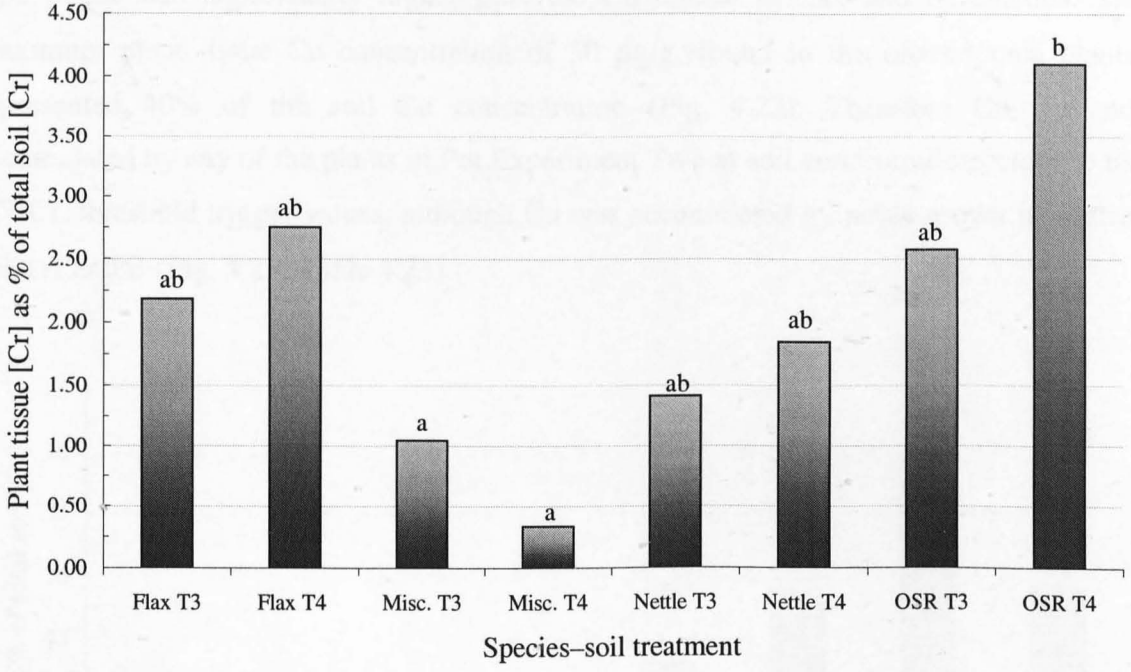

Figure 4.22 Stem and leaf tissue Cr concentrations of plant species grown in T3 and T4 soils. Plant tissue Cr concentration expressed as a % of soil total Cr concentration (Table 4.20, 4.24) for flax, miscanthus (Misc.), nettle and oilseed rape (OSR). Bars sharing the same letter are not significantly different (*t* test using Bonferroni correction, $P < 0.05$).

Oilseed rape and flax had the highest tissue Cr concentrations (Table 4.24). The greatest uptake of Cr was by the oilseed rape plants grown in the Cr_{T4} soil. The oilseed rape–T4 plants had a mean tissue concentration of 35 μg/g Cr, amounting to 4% of the soil Cr concentration, however, due to the sample variability, the Cr uptake in the oilseed rape–T4 system was only significantly different from the Cr uptake in miscanthus. Of the six metals in the study, Cr, had the lowest uptake from the T3 and T4 soils to the plant tissue in all the plant species studied, with respect to the soil total metal concentration (Fig. 4.22).

Chromium was therefore considered the least mobile of the metals for all of the soil–plant systems at concentrations around the ICRCL threshold trigger values.

Copper

The oilseed rape plants in the Cu_{T3} soil had the highest Cu tissue concentration of all of the plants grown in the Cu treated soils. The oilseed rape plant tissue Cu concentration in the Cu_{T3} soil, at 50 μg/g, was 29% higher than the next most Cu concentrated plant tissue, oilseed rape grown in the Cu_{T4} soil (Table 4.24; Fig. 4.23). The oilseed rape–Cu_{T3} and –Cu_{T4} systems were the only systems in Pot Experiment Two where the tissue concentration in the plant species–T3 soil exceeded the tissue concentration in the plant species–T4 soil, however, none of the T3 and T4 soils gave differences in tissue metal concentrations which were significantly different within plant species. The uptake of Cu in the nettle and oilseed rape plants was significantly higher than the Cu uptake in flax and miscanthus. The maximum plant tissue Cu concentration of 50 μg/g, found in the oilseed rape plants, represented 40% of the soil Cu concentration (Fig. 4.23). Therefore Cu was not accumulated by any of the plants in Pot Experiment Two at soil concentrations close to the ICRCL threshold trigger values, although Cu was accumulated by nettle grown in control soils A and B (Fig. 4.23; Table 4.23).

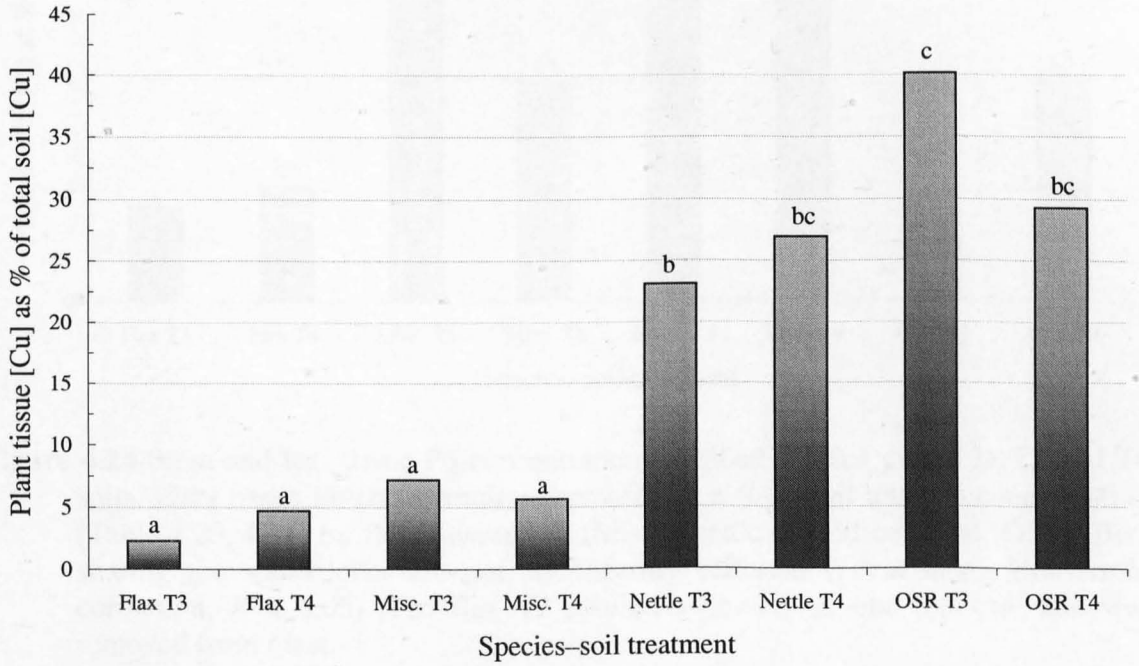

Figure 4.23 Stem and leaf tissue Cu concentrations of plant species grown in T3 and T4 soils. Plant tissue Cu concentration expressed as a % of soil total Cu concentration (Table 4.20, 4.24) for flax, miscanthus (Misc.), nettle and oilseed rape (OSR). Bars sharing the same letter are not significantly different (*t* test using Bonferroni correction, $P < 0.05$).

Lead

The variability in the concentrations of replicates meant that the differences observed between treatments were not significant except the nettle–Pb_{T3} and the oilseed rape–Pb_{T3} (Table 4.24). Nettle had the highest tissue Pb concentration of all the plants species grown in the Pb_{T3} and Pb_{T4} soils. The maximum mean tissue concentration, 107 μg/g for the nettle plants grown in the Pb_{T3} soil, was 20% of the soil Pb concentration (Fig. 4.24). Nettles grown in the T4 soil had a tissue concentration of 14% of the soil total Pb concentration which matched the Pb uptake by miscanthus grown in the T3 soil, with respect to the soil total Pb concentration. Lead was the only metal for which miscanthus achieved tissue metal concentrations greater than or equal to those of flax and oilseed rape. The three remaining treatments, flax grown in the T3 and T4 soils and oilseed rape in the T3 soil, all had tissue Pb concentrations of < 6% of the soil total Pb concentration making metal uptake in these systems among the lowest from the T3 and T4 soils.

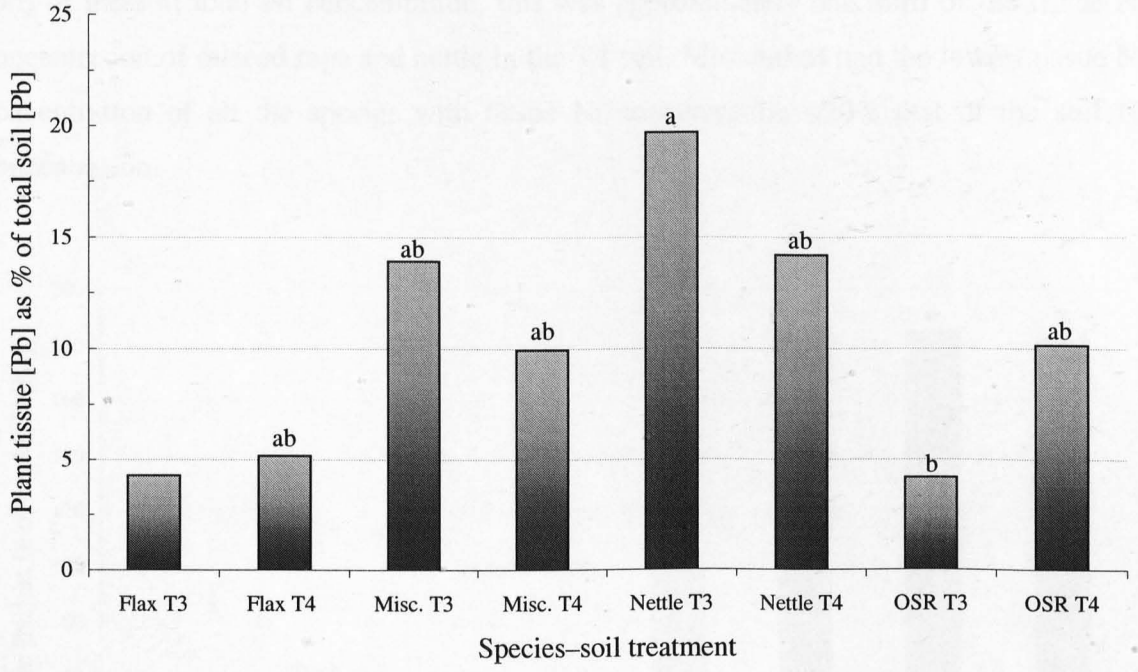

Figure 4.24 Stem and leaf tissue Pb concentrations of plant species grown in T3 and T4 soils. Plant tissue Pb concentration expressed as a % of soil total Pb concentration (Table 4.20, 4.24) for flax, miscanthus (Misc.), nettle and oilseed rape (OSR). Bars sharing the same letter are not significantly different (*t* test using Bonferroni correction, $P < 0.05$). The flax–T3 result was based on one replicate and was removed from *t* test.

Nickel

For all the plant species in Pot Experiment Two, the tissue Ni concentration was greater in the plants grown in the T4 soil than those grown in the T3 soil (Table 4.24) although this difference was only significant for nettles. Both oilseed rape and nettle had similar tissue Ni concentrations in the T4 soil of ~ 188 μg/g, the highest Ni tissue concentration in the experiment. The soil total Ni concentration of the Ni_{T4} soil was 103 μg/g (Table 4.20), thus the tissue concentrations of Ni in oilseed rape and nettle grown in the T4 soils showed a Ni accumulation of 180% of the soil total Ni concentration (Fig. 4.25). Nickel was extracted from the soil by the plant species and accumulated in the above ground plant tissues of oilseed rape grown in the T3 and T4 soil treatments and in the nettles grown in the T4 soil treatment. Nettles grown in the Ni_{T3} soil had a tissue concentration of 100% of the soil total Ni concentration so plants in this treatment did not accumulate Ni although they extracted more Ni from the soil than the flax and miscanthus plants (Fig. 4.25). Flax grown in the Ni treated soils had tissue Ni concentrations between 47% (T3 soil) and 55% (T4 soil) of the soil total Ni concentration, this was approximately one third of the tissue Ni concentration of oilseed rape and nettle in the T4 soil. Miscanthus had the lowest tissue Ni concentration of all the species with tissue Ni concentration <20% that of the soil Ni concentration.

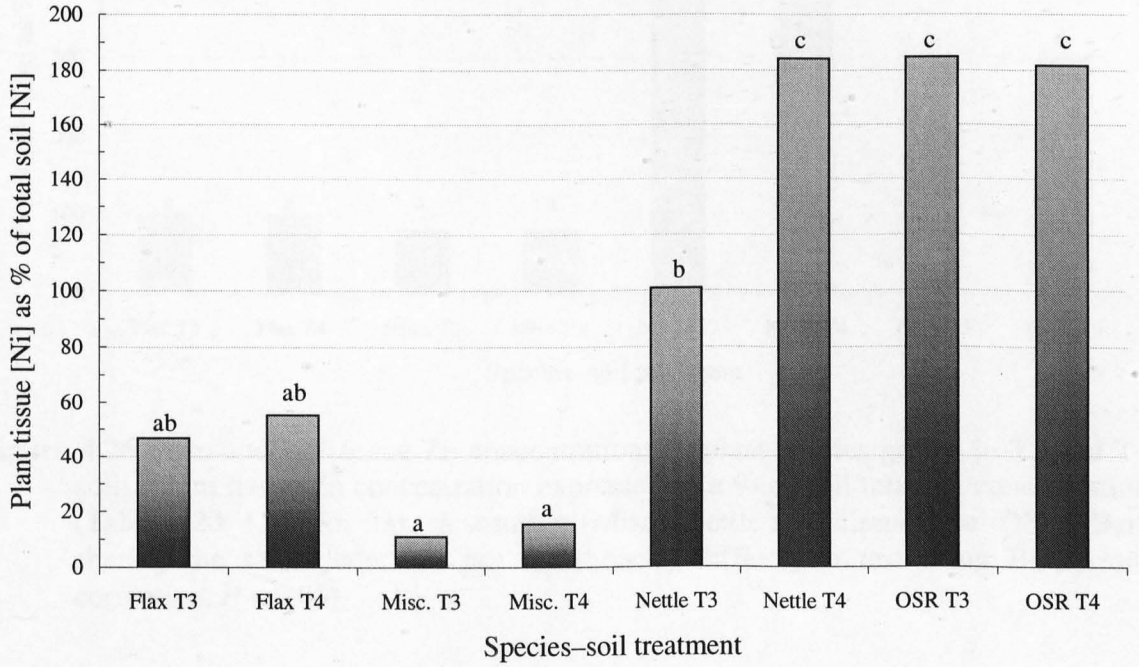

Figure 4.25 Stem and leaf tissue Ni concentrations of plant species grown in T3 and T4 soils. Plant tissue Ni concentration expressed as a % of soil total Ni concentration (Table 4.20, 4.24) for flax, miscanthus (Misc.), nettle and oilseed rape (OSR). Bars sharing the same letter are not significantly different (*t* test using Bonferroni correction, $P < 0.05$).

Zinc

As was observed in the Cu treatments, the uptake of Zn by the plant species fell into two groups; lower Zn uptakes were observed for miscanthus and flax compared to the higher uptakes observed for nettle and oilseed rape (Table 4.24). Nettle plants had the highest tissue Zn concentration of all the plant species in Pot Experiment Two. Nettle plants grown in the T4 soil accumulated 1937 μg/g Zn in their tissue, an accumulation of 587% of the soil total Zn concentration (Fig. 4.26). Furthermore, the nettle plants in the T4 soil accumulated almost twice as much Zn as the second highest accumulator in the T4 soil, oilseed rape. Nettle plants grown in T4 soils had significantly higher tissue Zn concentrations than the plants in the T3 soil. Additionally, the nettle and oilseed rape plants grown in the T4 soil accumulated more Zn in proportion to the soil Zn concentration than the plants grown in the T3 soil. In the case of nettle, the increase in Zn content from the Zn_{T3} to Zn_{T4} soil had a significant effect on the accumulation of Zn by this plant species.

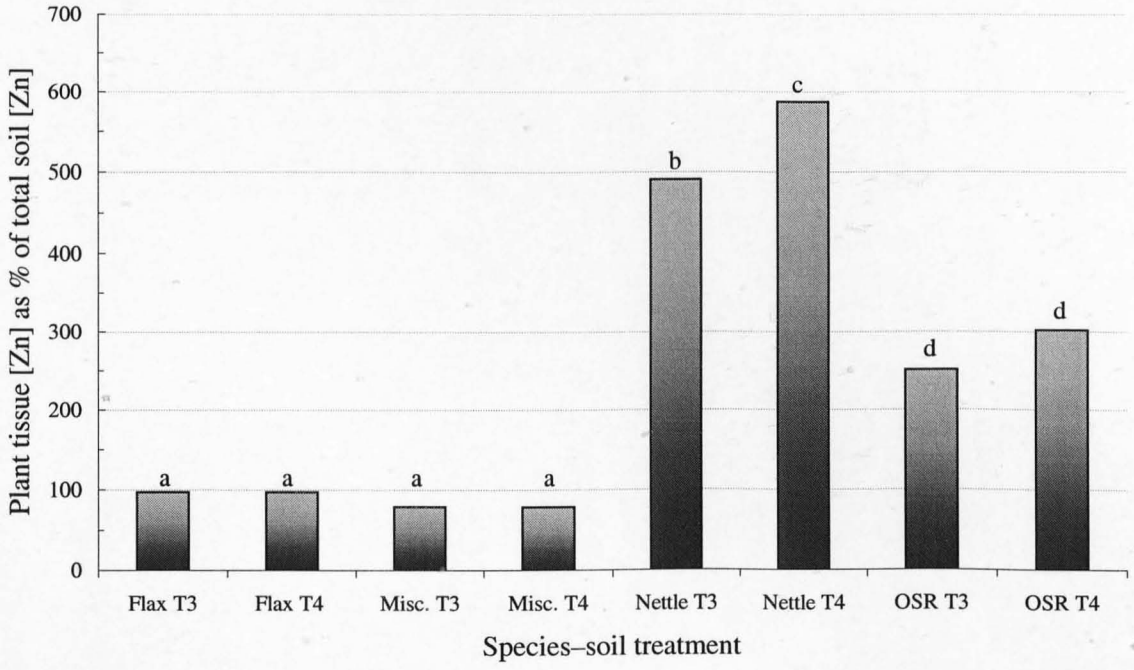

Figure 4.26 Stem and leaf tissue Zn concentrations of plant species grown in T3 and T4 soils. Plant tissue Zn concentration expressed as a % of soil total Zn concentration (Table 4.20, 4.24) for flax, miscanthus (Misc.), nettle and oilseed rape (OSR). Bars sharing the same letter are not significantly different (*t* test using Bonferroni correction, $P < 0.05$).

For flax, miscanthus and oilseed rape, the observed rise in tissue concentration between the T3 and T4 soils was not significant (Table 4.24) indicating that the differences in soil concentrations were not sufficient to elicit a detectable response in uptake. For these plants there was no difference in the proportion of the soil total Zn concentration removed from the soil by the plants grown in either treatment (Fig. 4.26). Neither the miscanthus nor the flax plants accumulated Zn, with their plant tissue concentrations between 79% and 97% of the soil total Zn concentration. In miscanthus, the tissue Zn concentration was the higher than for any other metal taken up by miscanthus (Table 4.24). Zinc was also one of the two most mobile metals with respect to the soil total metal concentration (Fig. 4.21–4.26).

4.2.3.3 Sewage sludge soil

Studying the above ground tissue concentration of the six metals in the plant species–sewage sludge soil system allowed the behaviour of the metals in a heavily contaminated soil with a high organic matter content to be elucidated. Soil total metal concentrations for the sewage sludge soil were detailed in Section 4.2.1.3.

Cadmium

The sewage sludge soil had the greatest soil total Cd concentration (44 μg/g) of all the Pot Experiment Two soils (Table 4.19–4.21). Despite the sewage sludge soil Cd loading, approximately 10 fold that of Cd_{T4}, the plants growing in the sewage sludge soil did not accumulate Cd (Table 4.26). Oilseed rape plants had the greatest tissue Cd concentration (4.82 μg/g) of all the plants grown in the sewage sludge soil (Table 4.25), but this represented a tissue concentration only ~ 10% of the soil total Cd concentration (Table 4.26), a marked decrease from the accumulation of >1000% seen in flax and oilseed rape plants growing in the Cd_{T3} and Cd_{T4} soils (Fig. 4.21). This decrease in mobility highlighted the impact the matrix properties had on the mobility of the metals between the soil–plant systems. Although Cd was not taken up from the sewage sludge soil to the same extent as from the T3 and T4 soils, Cd gave the highest mobility in the plant–sewage sludge soil systems (oilseed rape–sewage sludge soil system) equal with Ni (nettle–sewage sludge soil system) (Table 4.26).

Table 4.25 Tissue metal concentrations of plants grown in the sewage sludge soil. Mean plant tissue metal concentrations (μg/g) for: flax, miscanthus, nettle and oilseed rape. Values are calculated from four replicate pots (n=4). L.D. denotes plant tissue metal concentrations below the limit of detection.

Plant species	Cd		Cr		Cu	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Flax	4.24	*0.41*	L.D.		1.58	*1.83*
Miscanthus	1.87	*1.28*	L.D.		2.91	*n=1*
Nettle	2.33	*0.19*	L.D.		38.44	*4.36*
Oilseed rape	4.82	*0.82*	8.57	*n=1*	25.54	*3.23*
	Pb		Ni		Zn	
	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*	(μg/g)	*st. dev.*
Flax	L.D.		3.82	*0.41*	62.30	*9.92*
Miscanthus	L.D.		9.82	*8.58*	100.89	*126.33*
Nettle	L.D.		51.76	*10.91*	44.68	*4.70*
Oilseed rape	L.D.		43.50	*6.36*	150.97	*45.27*

Chromium

Of the four plant species grown in the sewage sludge soil only, oilseed rape tissue contained detectable quantities of Cr (Table 4.25); this was only ~ 1% of the soil total Cr concentration (Table 4.26), despite a soil Cr loading of > three times the Cr ICRCL threshold trigger value (Table 4.21).

Table 4.26 Uptake of metals by plants grown in the sewage sludge soil. Values shown are the mean sewage sludge soil plant tissue metal concentrations expressed as a percentage of the soil total metal concentrations. Tissue concentrations < 0.5% are denoted by "†"; below the detection limit are denoted by "-".

Plant species	Cd	Cr	Cu	Pb	Ni	Zn
Flax	10	-	†	-	1	3
Miscanthus	4	-	†	-	2	5
Nettle	5	-	4	-	11	2
Oilseed rape	11	†	3	-	9	8

The limit of detection indicates that Cr is present at <1% of the soil total Cr concentration whilst Pb is present at <3% of the soil total Pb concentration.

Copper

Miscanthus, flax and oilseed rape grown in the sewage sludge soil, which had a soil Cu concentration ~ seven fold more than the Cu ICRCL threshold trigger value (Table 4.21), had lower Cu tissue concentrations than the plants grown in the T3 and T4 soils (Table 4.24, 4.25). Flax plants grown in the sewage sludge soil, the plant species with the lowest tissue Cu concentration, had a similar tissue Cu concentration to the flax plants grown in the control soils (Table 4.22, 4.25). Nettle, which had the highest tissue Cu concentration, was the only plant species whose tissue concentration was greater in the sewage sludge soil than in the T3 and T4 soils, albeit marginally. The mobility of Cu in the sewage sludge soil (Table 4.26) was > 10 fold less than Cu mobility in the T3 and T4 soils (Fig. 4.23) for all the plant species except nettle where the mobility in the sewage sludge soil was only ~ six fold less.

Lead

The sewage sludge soil had a soil Pb concentration of 1.5 times the Pb ICRCL threshold trigger value (Table 4.21) yet none of the plants grown in this soil had any detectable Pb in their tissues (Table 4.25). Lead was the only metal which was not taken up into plant tissues in detectable quantities although this metal has the highest detection limit of the metals in the study (Section 2.12.4; Table 2.14).

Nickel

The Ni loading of the sewage sludge soil was seven fold greater than the Ni ICRCL threshold trigger value (Table 4.21), however, the tissue Ni concentrations in the plants grown in the sewage sludge soil were up to 14 fold less than the tissue Ni concentrations of the plants grown in the Ni_{T4} soils (Table 4.24, 4.25). The exception to this was miscanthus for which the plant tissue concentration taken up from the sewage sludge soil was equal to that taken up from the Ni_{T3} soil. In the sewage sludge soil–nettle system, Ni was the most mobile of the six metals studied and overall, this system was equal with Cd in the sewage sludge soil–oilseed rape system in producing the two most mobile metals in the sewage sludge soil–plant systems (Table 4.26).

Zinc

The sewage sludge soil had a soil Zn concentration > six times the Zn ICRCL threshold trigger value (Table 4.21). Despite the high Zn loading of the sewage sludge soil, the uptake of Zn by the plants in the study was low at < 9% of the soil total Zn concentration (Table 4.26). The tissue Zn concentrations of the plant species grown in the sewage sludge soil were all lower than the tissues of those species grown in the Zn_{T3} and Zn_{T4} soils (Table 4.24, 4.25). The highest uptake of Zn from the sewage sludge soil was recorded in oilseed rape at 151 μg/g which was the highest tissue metal concentration recorded for plants grown in the sewage sludge soil (Table 4.25). Zinc was the metal present at the highest concentration in flax, miscanthus, and oilseed rape tissues. The high tissue Zn concentrations were offset by the high Zn loading of the sewage sludge soil and therefore Zn was observed to be the most mobile metal in the miscanthus–sewage sludge soil system only (Table 4.26).

4.2.3.4 Metal uptake summary

Of all the metals in the study, the highest tissue concentrations were seen in plants grown in the Zn_{T3} and Zn_{T4} soils, nettles in the Zn_{T4} soil had the highest plant tissue metal concentration recorded in Pot Experiment Two (1937μg/g, Table 4.24). In terms of the proportion of the soil metal accumulated by the species in Pot Experiment Two, the flax and oilseed rape–Cd_{T3} and –Cd_{T4} soil systems produced the highest tissue concentrations with respect to the soil total metal concentration, with all four systems having tissue Cd concentrations greater than 1000% of the soil Cd concentration. The lowest tissue concentrations were recorded for Cr and the plants grown in the Cr soils also had the lowest tissue concentrations when expressed as a percentage of soil total metal concentration. The low uptake of Cr was in agreement with the low mobility of Cr observed in the T1 and T2 soils (the parent soils of the T3 and T4 soils), as well as the sewage sludge soil, using $CaCl_2$ and EDTA extractant solutions. Cadmium, Ni and Zn were the only metals where the plant tissue metal concentrations exceeded the soil metal concentrations, indicating metal accumulation.

Nettle not only had the greatest tissue metal concentration in the Zn_{T4} soil, it was also the plant species which had the highest tissue concentration for Pb and Ni although oilseed rape plants in the Ni soils had similar tissue Ni concentrations. In the Cd, Cr and Cu soils, oilseed rape plants had the highest tissue metal concentrations of all the plant species in the experiment. For four of the six metals (Cd, Cr, Ni and Zn) the poorest uptake of metal to plant tissue was recorded for miscanthus, while in the remaining two soils (Cu and Pb) miscanthus plants only had a plant tissue metal concentration greater than flax.

For each plant, the order of mobility of the metals was seen by ranking the tissue metal concentrations expressed as a percentage of the soil metal concentration. These percentages were ranked as follows, the highest mobility appearing on the left:

Miscanthus: $Zn \geq Cd \gg Ni = Pb > Cu > Cr$

Flax: $Cd \gg Zn \gg Ni \gg Pb \geq Cu \geq Cr$

Oilseed rape: $Cd \gg Zn \gg Ni \gg Cu \gg Pb > Cr$

Nettle: $Zn \gg Cd \gg Ni \gg Cu > Pb \gg Cr$

It can be seen from the ranking of the metals (above) that the metals fell into a similar order across each plant species with Cr, Cu and Pb less mobile than Cd, Ni and Zn. However, the extent of the mobility of the metals and the precise order of rankings varied according to the plant species in question. Most notably, the behaviour of Pb and Ni in the miscanthus showed the Pb tissue concentration of miscanthus, expressed as a percentage of the soil concentration, to be similar to that of Ni, whereas in the other plants in the study, Ni was present in plant tissue at a considerably higher percentage of the soil Ni concentration than Pb. Furthermore, the behaviour of Ni indicated the difficulty of making generalised statements regarding the mobility of metals within soil plant systems. Nickel was a metal which was accumulated by two of the four plant species under investigation, however, simultaneously Ni was a metal whose mobility in miscanthus was similar to the mobility of Pb, a metal normally considered as having low mobility (Lasat, 2002).

For each of the metals in the study, the relative suitability of the four plant species with respect to each other was determined by ranking the plant tissue metal concentration (μg/g) of the plants grown in the T3 and T4 soils (Table 4.24). These were ranked as follows, the highest tissue concentration appearing on the left:

Cadmium: OSR–T4 > Flax–T4 > OSR–T3 ≥ Flax–T3 ≫ Nettle ≫ Misc.

Chromium: OSR–T4 > Flax–T4 > OSR–T3 > Flax–T3 > Nettle >Misc.

Copper: OSR> Nettle ≫ Misc. > Flax

Lead: Nettle > Misc.–T3 ≥ OSR–T4 ≥ Misc.–T4 ≫ Flax > OSR–T3

Nickel: OSR ≥ Nettle–T4 ≫ Nettle–T3 > Flax ≫ Misc.

Zinc: Nettle > OSR > Flax > Misc.

The plant species rankings (above) showed that oilseed rape was the species which had the highest tissue concentrations for four (Cd, Cr, Cu and Ni) of the six metals studied, although for nickel, the tissue concentrations in nettle were similar to those of oilseed rape. The two remaining metals (Pb and Zn) had the highest tissue concentration in nettle. Nettle also had the second highest tissue concentrations of Cu and Ni. On the basis of above ground plant tissue concentrations, oilseed rape and nettle were the most promising phytoremediator candidates.

In the sewage sludge soil, with its high concentration of all the metals in the study, it was consistently seen that the plant tissue concentration of each metal, was lower than that of

the plants grown in the corresponding contaminated soils. This behaviour was also seen in the control soils. Control soil C, despite having a higher soil concentration of all the metals studied than the control soils A and B, gave plant tissue concentrations lower than control soils A and B (with the exception of Cd and Ni uptake by miscanthus in control soil C). The sewage sludge soil and the Garscube control soil C were both soils with a high organic matter content; the reduced uptake of metals from these soils compared to the artificially made contaminated and control soils indicated that the organic matter content of the contaminated soil may be a key factor in the mobility of the metals in the soil–plant system.

4.2.4 Total quantities of metal removed from the soil

The goal of phytoremediation is to extract as much of the polluting metal from the soil as possible. It is therefore essential when evaluating potential phytoremediators to establish plant species capable of high metal uptake coupled with high biomass production. The product of the plant tissue metal concentration (Section 4.2.3) and the yield of plant tissue (Section 4.2.2.2) gave the total quantity of metal removed from the soil matrix (Fig. 4.27–4.32). Each of the metals were considered in turn before summarising the findings.

4.2.4.1 Cadmium total uptake

Miscanthus was previously reported to give the highest yield of plant tissue in the Cd treated soils (Section 4.2.2.2). Flax and oilseed rape were found to contain the highest tissue Cd concentrations (Section 4.2.3.2), however, flax was the plant species which extracted the greatest quantity of Cd (68 μg in Cd_{T4}) from the soil matrix (Fig. 4.27). This was attributable to a flax tissue concentration ten times greater than that of miscanthus and a flax yield more than double that of oilseed rape. Despite having the poorest Cd uptake, miscanthus, owing to its high yield relative to the other plant species, was able to extract more Cd from the treated soils than the oilseed rape and nettle plants (Fig. 4.27).

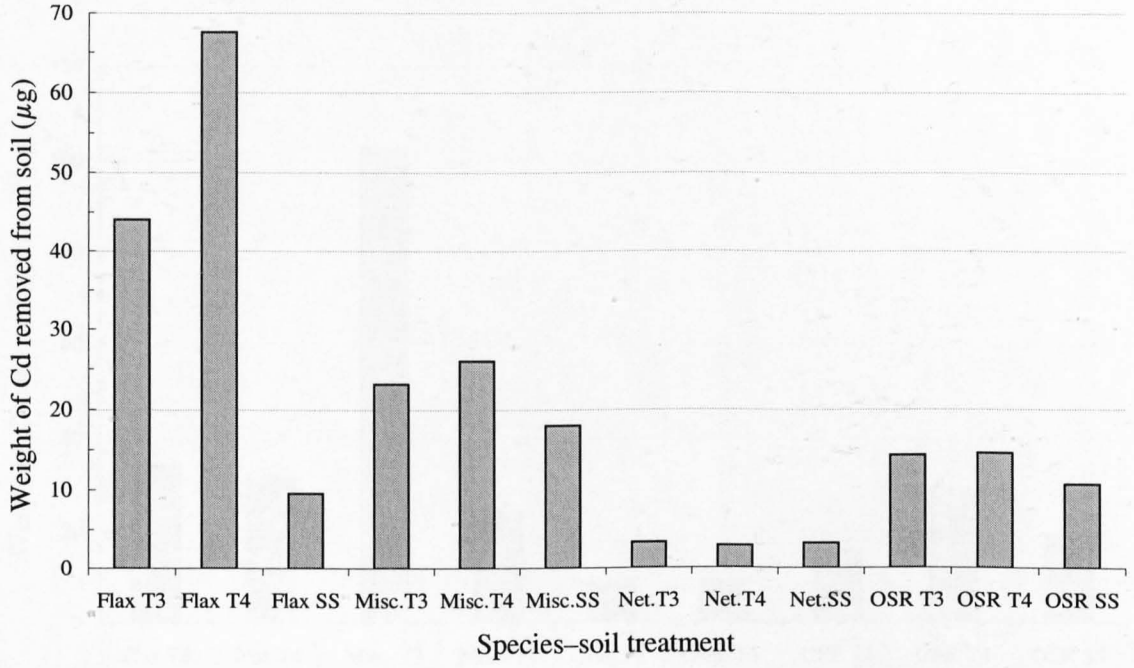

Figure 4.27 Total weight of Cd removed from the T3 and T4 artificial soils and sewage sludge soil (SS) by each plant species. Weight expressed as μg removed from soil by flax, miscanthus (Misc.), nettle (Net.) and oilseed rape (OSR).

For flax, miscanthus and oilseed rape, less Cd was taken up from sewage sludge soil than from the Cd_{T3} and Cd_{T4} soils, despite the sewage sludge having a soil Cd concentration ~10 times higher than the Cd_{T3} and Cd_{T4} soils. Only nettle plants removed as much Cd from the sewage sludge soil as from the Cd_{T3} and Cd_{T4} soils and this was due to the nettle plants grown in the sewage sludge having a yield > four times higher than the Cd_{T3} and Cd_{T4} soil yields.

4.2.4.2 Chromium total uptake

The greatest quantity of Cr (103 μg) was removed from the contaminated soils by miscanthus grown in the Cr_{T3} soil (Fig. 4.28). Although oilseed rape, flax and nettle all had higher tissue Cr concentrations than miscanthus (Section 4.2.3.2; Fig. 4.22), the higher yield of miscanthus compensated for the lower uptake (Section 4.2.2.2), resulting in the greater remediation of the Cr contaminated soil by miscanthus observed. The miscanthus plants in the T3 soil removed three times as much Cr as the next most effective plant–soil system (flax–T4). In the T4 soil, a poorer yield of miscanthus accompanied by a poorer uptake of Cr from the soil meant that both flax and oilseed rape were able to remove more Cr from the T4 soil than miscanthus (Fig. 4.28).

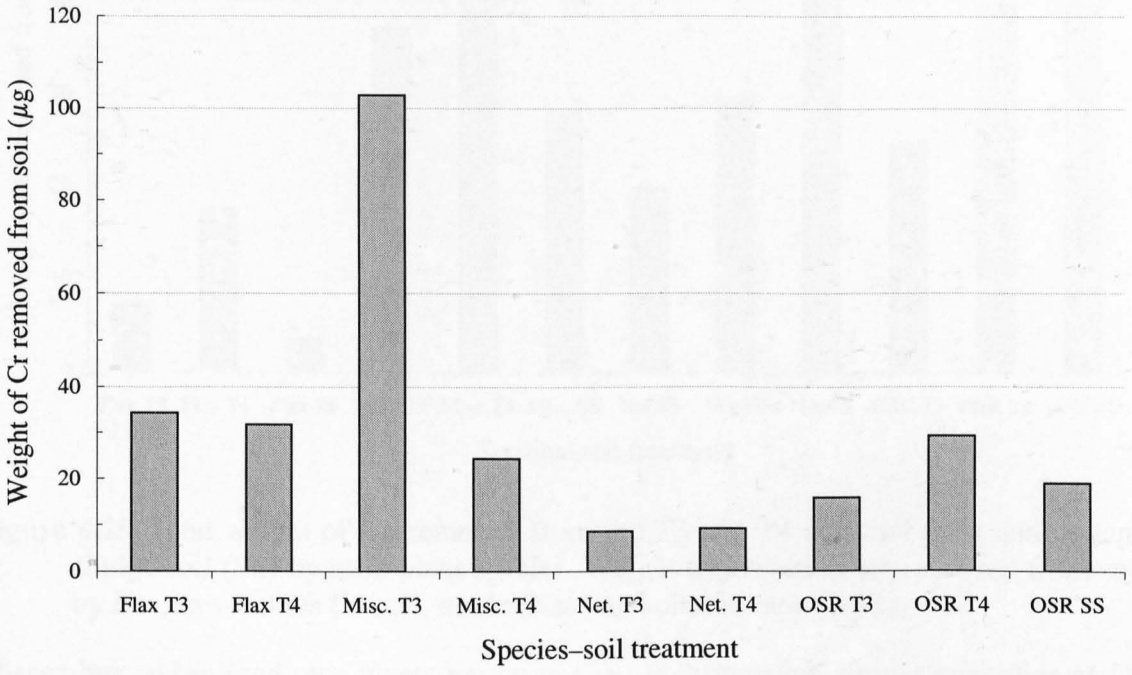

Figure 4.28 Total weight of Cr removed from the T3 and T4 artificial soils and sewage sludge soil (SS) by each plant species. Weight expressed as μg removed from soil by flax, miscanthus (Misc.), nettle (Net.) and oilseed rape (OSR).

Oilseed rape was the only plant species able to extract a detectable quantity of Cr from the sewage sludge soil due to the low mobility of Cr in the sewage sludge soil (Table 4.8).

4.2.4.3 Copper total uptake

Copper, the most mobile metal in the EDTA-extractable fraction of the sewage sludge soil (Table 4.9), was extracted at higher quantities from the sewage sludge soil than the Cu_{T3} and Cu_{T4} soils by both oilseed rape and nettle (Fig. 4.29). Uptake of Cu from sewage sludge by flax and miscanthus was lower than for the Cu_{T3} and Cu_{T4} soils. The greater uptake by nettle and oilseed rape was due to the greater yield of oilseed rape and nettle biomass in the sewage sludge soil compared to the T3 and T4 soils.

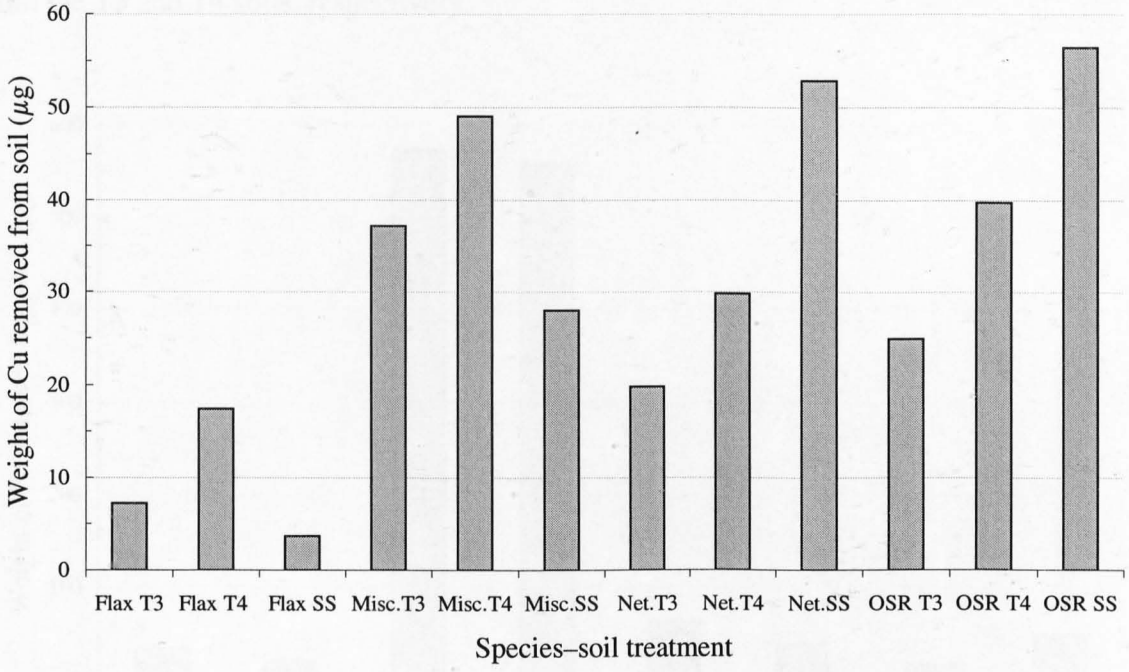

Figure 4.29 Total weight of Cu removed from the T3 and T4 artificial soils and sewage sludge soil (SS) by each plant species. Weight expressed as μg removed from soil by flax, miscanthus (Misc.), nettle (Net.) and oilseed rape (OSR).

Miscanthus and oilseed rape plants grown in Cu_{T4} both removed similar quantities of Cu from the soil, 49 μg and 40 μg respectively (Fig. 4.29). The total uptake of Cu by miscanthus grown in the T4 soil was calculated using the yield of the original plants (6.63 g) grown in this treatment rather than the yield of the replacement plants (1.89 g) (Section 2.7.2). Of all the treatments in the study the Cu treated soils gave the lowest yields of miscanthus (Section 4.2.2.2). In contrast, the oilseed rape grown in the Cu_{T4} treatment gave a higher yield than any of the other oilseed rape–metal soils, with the exception of the Zn soils. Although miscanthus yield in the Cu soils was so poor, the miscanthus plants grown in the Cu soils had a small yield advantage over other plant species in the study. Miscanthus had a yield around six times that of oilseed rape, whereas oilseed rape had a tissue Cu concentration 5.2 fold higher than that of miscanthus. The resulting remediation by both miscanthus and oilseed rape was similar despite the contrasting mechanisms of Cu removal.

4.2.4.4 Lead total uptake

Lead was the only metal where the miscanthus tissue metal concentration was greater than or equal to the tissue concentrations of flax and oilseed rape in T3 and T4 soils (Section 4.2.3.2). Only nettle plants had a greater tissue Pb concentration than miscanthus (Table 4.24). The combination of miscanthus having a greater uptake of Pb than the other plants, except nettle, accompanied by its high yield, resulted in the total quantity of Pb removed from the soil by miscanthus being nine times greater than oilseed rape (T4), the next most effective Pb remediating plant (Fig. 4.30). Miscanthus removed 574 μg and 559 μg of Pb from the T3 and T4 soils, respectively.

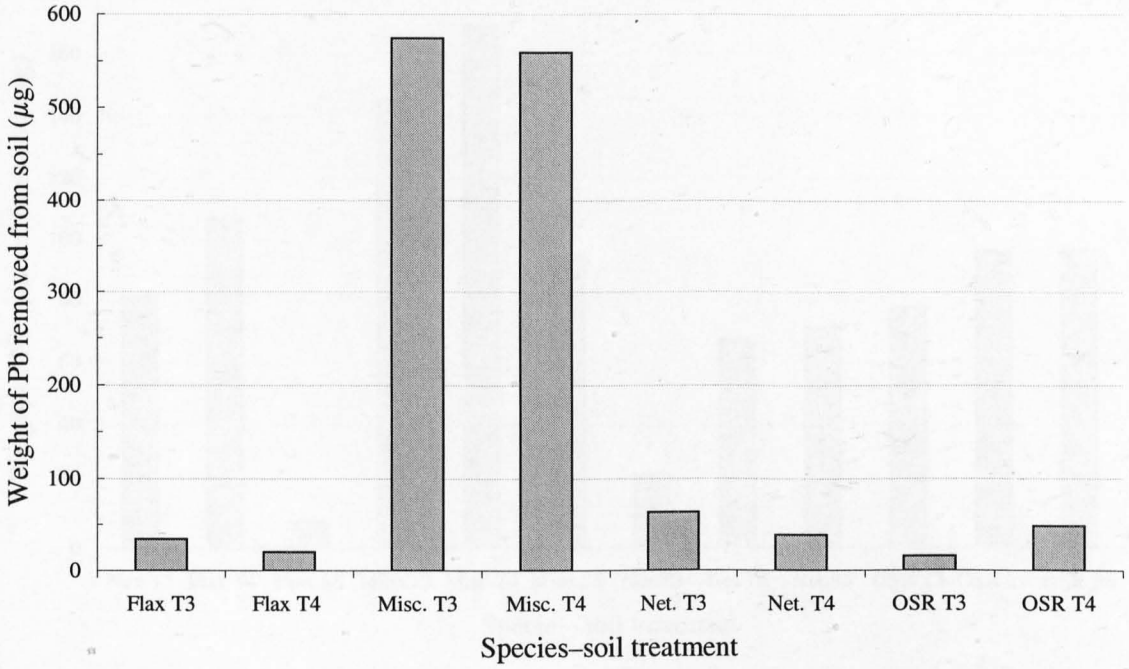

Figure 4.30 Total weight of Pb removed from the T3 and T4 artificial soils by each plant species. Weight expressed as μg removed from soil by flax, miscanthus (Misc.), nettle (Net.) and oilseed rape (OSR).

Lead tissue concentrations in all species grown in sewage sludge soils were below the limit of detection and thus the total weight of Pb removed was not quantifiable.

4.2.4.5 Nickel total uptake

Miscanthus removed the greatest quantity of Ni from both the Ni_{T3} and Ni_{T4} soils (Fig. 4.31), despite having the lowest tissue Ni concentrations of all the plant species. Again, miscanthus' ability to remove the most metal from the soil was a result of its high biomass. Oilseed rape and nettle, the plants with the highest tissue Ni concentration, had the lowest yields of the plants grown in the Ni soils. As a result flax plants, whose tissue Ni concentrations were between one third and a half that of oilseed rape and nettle, removed more metal than the oilseed rape and nettle plants.

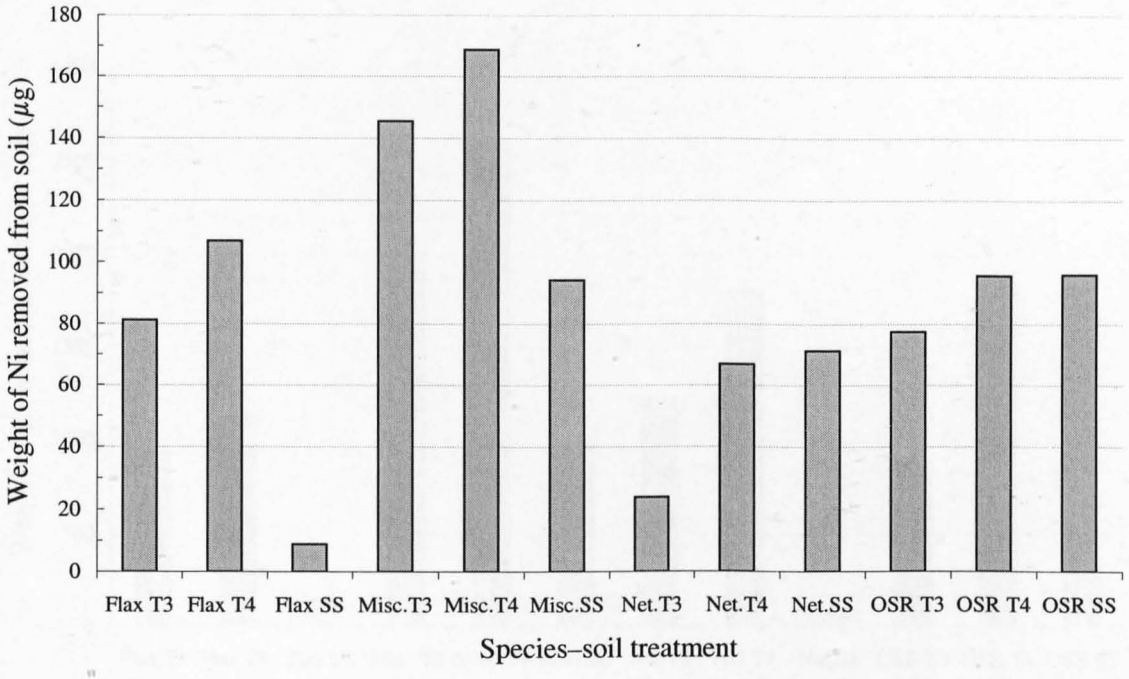

Figure 4.31 Total weight of Ni removed from the T3 and T4 artificial soils and sewage sludge soil (SS) by each plant species. Weight expressed as μg removed from soil by flax, miscanthus (Misc.), nettle (Net.) and oilseed rape (OSR).

Removal of Ni from the sewage sludge soil by flax and miscanthus was lower than from the T3 and T4 soils due to the low tissue Ni concentration of these plant species. Both oilseed rape and nettle had a higher uptake of Ni grown in sewage sludge soil than flax and miscanthus, although the tissue Ni concentration in the plants grown in the sewage sludge soil was lower than those grown in the T3 and T4 soils. The yield achieved meant that the total Ni removed from sewage sludge soil by nettle and oilseed rape, was similar to that removed from the T4 soils.

4.2.4.6 Zinc total uptake

The highest quantity of Zn was removed from the contaminated soils by miscanthus (Fig. 4.32). Miscanthus grown in the Zn_{T4} removed 2601 μg of Zn from the soil, the largest amount of metal removed by any of the plant–metal combinations and 737 μg more Zn than was removed by oilseed rape in the Zn_{T4} soil, the next best Zn remediator. Miscanthus was again able to remove the highest quantity of Zn, despite having the lowest tissue Zn concentration (Section 4.2.3.2), as a result of its high biomass (Section 4.2.2.2). Nettle, which had the highest tissue Zn concentration of all the plants (Section 4.2.3.2) was able to remove more Zn than the flax plants and oilseed rape grown in the Zn_{T3} soil.

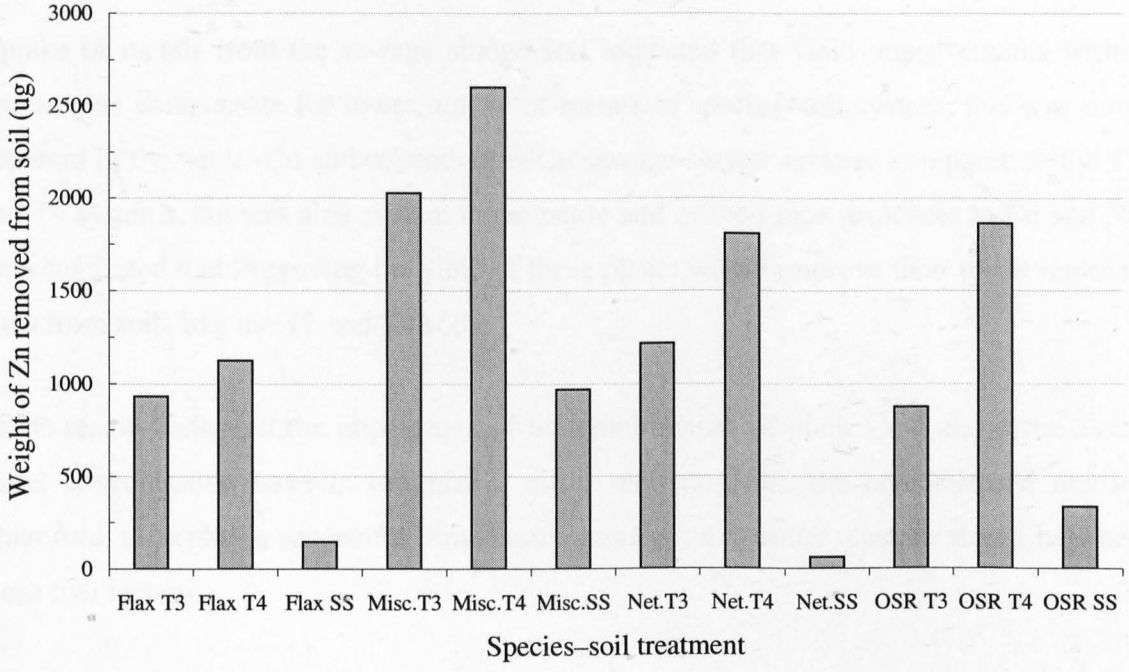

Figure 4.32 Total weight of Zn removed from the T3 and T4 artificial soils and sewage sludge soil (SS) by each plant species. Weight expressed as μg removed from soil by flax, miscanthus (Misc.), nettle (Net.) and oilseed rape (OSR).

The low tissue Zn concentration of the plants grown in the sewage sludge soil was reflected in the low total Zn removal from the sewage sludge soil by the each of the plant species compared to the T3 and T4 soils.

4.2.4.7 Total uptake summary

Each of the metals in the study was removed in the greatest quantity from the contaminated soils by miscanthus, with the exception of Cd. For Cr, Ni and Zn, miscanthus had the lowest tissue metal concentrations of all the plant species, while Cu tissue concentrations were only lower in flax than miscanthus and Pb tissue concentrations were lower in flax and oilseed rape than miscanthus. Despite miscanthus having poor uptake of the metals compared to the other plant species it was able to remove more metal due to its high yield. Zinc was removed from the contaminated soils in the greatest quantities by all of the plant species in the experiment, although both Cr and Pb were present at higher soil concentrations.

Uptake of metals from the sewage sludge soil indicated that yield improvements within species can compensate for lower uptake of metals in species–soil system; this was most apparent in the nettle–Cu and oilseed rape–Cu sewage sludge systems compared to the T3 and T4 systems, but was also evident in the nettle and oilseed rape responses to Cd and Ni. This suggested that improving the yield of these plants would improve their metal removal rates from soils like the T3 and T4 soils.

These results indicated the importance of the combination of plant yield and plant tissue metal concentration have in optimising metal removal from the contaminated matrix. Therefore, to deploy a successful remediation strategy, a balance must be struck between these two factors.

5 Hydroponics

Hydroponics is the growth of plants in a medium which contains no soil. Several types of hydroponic growth systems are possible. In this chapter two techniques have been used: nutrient film technique (NFT) hydroponics (Section 5.1) and static hydroponics (Section 5.2). In both techniques, two key roles usually played by the soil matrix have been substituted for using soil free alternatives. Firstly, the nutrients needed by the plant, usually obtained from the soil matrix, were provided in the nutrient solution. Secondly, the structural support normally provided by the soil was provided by using growth collars (Section 2.8.2).

The static and NFT hydroponic techniques were distinctly different. In the static hydroponic technique, plant roots were immersed in the nutrient solution thus the solutions had to be agitated in order to aerate the nutrient solution. Therefore, the plant roots were subjected to a matrix which became increasingly anaerobic between agitations. By contrast, the principle of the NFT hydroponic technique was that the plant roots were simultaneously surrounded by nutrient solution and by air (Section 2.8.1). This was achieved by having a nutrient film sufficiently shallow that the root was not completely submerged in the solution. Furthermore, the solution itself was kept aerated by constant trickling through the system coupled with a high surface area in the NFT troughs.

Both systems allowed a study to be made of plant response to precisely controlled solution metal concentrations free from complications attributable to metal–soil interactions.

5.1 NFT study

A NFT system was used to establish the growth response of flax to a range of solution concentrations of Cd, Cr, Cu, Pb, Ni and Zn. This system gave an insight into the plants' tolerance of the contaminant metals independent of any soil conditions or other matrix interference. In the NFT system, constant flow of solution over the roots prevented the rhizosphere becoming anaerobic and maintained solution homogeneity through continuous mixing of the solution.

Of the four plant species: flax, miscanthus, nettle and oilseed rape, flax alone was considered in the NFT study; the variety Viking was used in the NFT study. It was not possible to grow miscanthus and nettle rhizome pieces in the NFT system using the method developed (Section 2.8), as there was insufficient contact between the large root systems of these plants and the thin film of metal solution in the NFT troughs. Additionally, the time required to establish an effective and reproducible NFT system and the pressure of other users for green house space, meant that there was insufficient time to study more than one plant species.

5.1.1 Method development

A NFT system must provide a steady flow of nutrient solution to plant roots of a depth shallow enough to prevent the roots becoming anaerobic, while ensuring an even distribution of the solution across the roots such that the roots do not dry out. Design of a NFT system incorporated measures to minimise algal growth as algae could remove nutrientsand possibly metals from the system in addition to promoting disease.

In the original design, large (~50-l) NFT reservoir tubs (Sunlighter Systems) supported the NFT channels. In practice the NFT reservoir tubs were unsuitable due to their large size. The size of the tubs meant solution evaporation was high due to the high surface area of the solution, and the pumps tended to run dry even when there was around 2 litres of solution left in the tub. The size also dictated that the channels had to be run in alternating directions, making the system awkward to work with. Additionally, using the tubs to support the channels was impractical as the system had to be completely dismantled every time the solutions were changed.

A metal frame was constructed (Handy Angles) to support the channels removing the need to support the trays on the reservoir tubs. The reservoir tubs were replaced by 5-l reservoir containers: these enclosed containers, in which the solution has a low surface area,

minimised solution evaporation (Section 2.8.1). The smaller size of the 5-l reservoir containers allowed the channels to be run in the same direction and simplified changing the solutions as replacement containers could be filled and transported to the greenhouse whilst the system was running.

Evaporation of the solution from the system remained problematic due to the high surface area and black colour of the channels. The dark colour of the channels resulted in the channels becoming warm due to efficient absorption of energy from light sources. Specialist plastic NFT sheeting was used to minimise evaporation problems in the NFT system (Section 2.8.2).

In an NFT system the plants are provided with nutrients from solution rather than soil. However, soil also provides structural support, a soil function which had to be recreated in the NFT system design. Avoidance of metal–matrix interactions was the reason for using a soil-less growth system, therefore, it was important that new support structure was as inert as possible. Rockwool slabs are a common support matrix used with NFT channels to provide an inert growing matrix with good water holding capacity and root aeration characteristics.

Rockwool slabs (Sunlighter Systems) were cut into slices in which the young seedling plants were germinated. Although the rockwool slices provided an effective support matrix, which had good contact with the nutrient solution, it was found unsuitable as both algae and fungi proliferated on the slices. An attempt was made to minimise the algal and fungal growth on the rockwool slices by partially covering the slices with NFT sheeting. Positioning the NFT sheeting was problematic and algal and fungal growth persisted at the base of the seedling stems, therefore, the rockwool slices proved unsuitable as a support matrix in the experiment. A method isolating the uppermost layer of the support matrix entirely from the nutrient solution was required to prevent algal and fungal growth.

Hydroponic growth collars

A container constructed using plastic drainpipe and ballet netting was used to retain a support matrix of inert plastic beads and vermiculite (Section 2.8.2), whilst allowing roots to grow through into the nutrient solution beneath.

The beads were chosen, for both static and NFT nutrient solution experiments, for two reasons: their black colour acted as an effective inhibitor of algal and fungal growth, and their size and weight did not inhibit root or shoot elongation. The beads alone, however, did not have sufficient water holding capacity to promote seed germination. A layer of vermiculite was included surrounding the seeds to stimulate germination. Thus a sandwich layer of vermiculite containing seeds between two layers of beads was used as an effective support matrix; the lower layer isolated the vermiculite from the NFT solution preventing the clay mineral adsorbing the metals and the upper layer prevented algal and fungal growth on the vermiculite seed bed (Fig. 5.1).

The collars were also advantageous as their uniform size and shape allowed holes which exactly fitted the external diameter of the collars, to be cut in a single piece of NFT sheeting. The whole NFT sheet was cut to exactly fit the internal size of the NFT tray. The sheeting was supported by a lip on the sides of the tray and by the clamp portion of the collars. The exact fit allowed the sheeting to be supported without coming into direct contact with the NFT solution. Both algal and fungal growth, and evaporation of solution was also prevented as the plastic sheeting was held firmly in place and covered the whole surface area of the NFT channel.

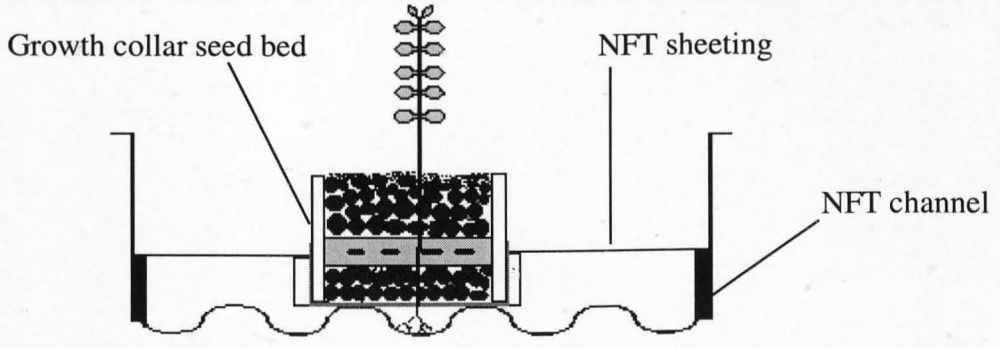

Figure 5.1 Hydroponic growth collar in the NFT tray. The cross-section view shows the growth collar seed bed supported on the ridged base of the NFT channel. The NFT sheeting is also shown suported by the collar and channel.

5.1.2 Flax in the NFT system

It was established in pot experiment 2 (Section 4.2.2) that the growth response of flax to elevated soil metal concentrations around the ICRCL threshold trigger levels was better than those of nettle and oilseed rape. Only miscanthus produced plants with greater height and yield than flax. Flax, an established fibre crop (Grieve, 1931; Ruckenbauer *et al.*, 2002), was a suitable plant species for the NFT study as large numbers of seeds were readily germinated (50 seeds sown per 70 mm diameter growth collar; Section 2.8.3), giving a rapid preparation time and good replication. Oilseed rape and nettle, also grown from small seeds, were suitable candidates for use in a NFT system. However, the broad leaves of the oilseed rape plants, prevented the same level of replication compared to flax in the growth collars, as overcrowding would have occurred. Nettle, unlike flax, was not considered in the germination study (Chapter 3) and thus was a less appropriate candidate than flax for the NFT study. Flax variety Viking was used in the NFT study.

5.1.3 NFT metal solution concentrations

Metal solutions were made up from their nitrate salts (Section 2.3). Each of the six metals was considered at six concentrations (Section 2.8.3; Table 2.10). The solution concentrations were chosen based on previous work conducted in the department (I. D. Pulford; personal communication).

All the metals were added at the same concentration on day 0 and day 7 with the exception of Zn. Zinc solutions concentration were increased by 40–65% in the second week. The Zn concentrations were increased as after the first week the plants growing in the most concentrated solutions did not show symptoms of metal toxicity to the same extent as the other metals in the study.

5.1.4 Metal removal from NFT solutions by flax

The NFT solution concentrations were measured at the start and end of the two 7-d periods (Fig. 5.2). These concentrations revealed the extent to which the plants removed the metals from the solution. The NFT system was constructed such that any loss of solution through evaporation was minimised, however, the solution volumes did decrease over the 7-d period due to evapotranspiration, and a small amount of evaporation. This decrease in volume would have resulted in an increased solution metal concentration. Changes in solution concentration were tempered by renewing the solutions half way through the 14-d growth period.

No increase in solution concentration was observed in any of the flax–metal systems, however, several flax–metal systems had no substantial reduction in metal solution concentration during the 7–14-d treatment period. These systems were flax grown in: 20 μg/ml Cd, 20 μg/ml Cu, 10 μg/ml Ni and 75 μg/ml Zn (Fig. 5.2).

The metal removed in the greatest quantity was Pb (Fig. 5.2d); more than 50 μg/ml was removed from the most concentrated Pb solution during both the 0–7-d and 7–14-d treatment periods, an amount equivalent to a removal of > 60% of the total solution metal. Lead and Cr were the only metals where a high percentage of total solution metal was removed during both the 0–7-d and 7–14-d treatment periods. The two most concentrated Ni solutions had the greatest difference in the proportion of metal removed between the 0–7-d and the 7–14-d treatment periods with ~ 40% less metal removed in the 7–14-d than the 0–7-d treatment period (Fig. 5.2e).

Chromium differed from the other metals in the study in that it was almost completely removed from solution at every concentration considered (Fig. 5.2b). However, the concentration of the Cr solutions was less than that of the other metals in the study by five times or more and therefore the quantity of Cr removed from the solutions was relatively low. Despite the use of phosphate free nutrient solution in the NFT study to minimise the precipitation of metals as insoluble salts (Huang *et al.*, 1996; Section 2.8.3), both the Cr and Pb may have been removed from solution by precipitation as a metal–root exudate complexes (Dushenkov *et al.*, 1995), rather than by plant uptake.

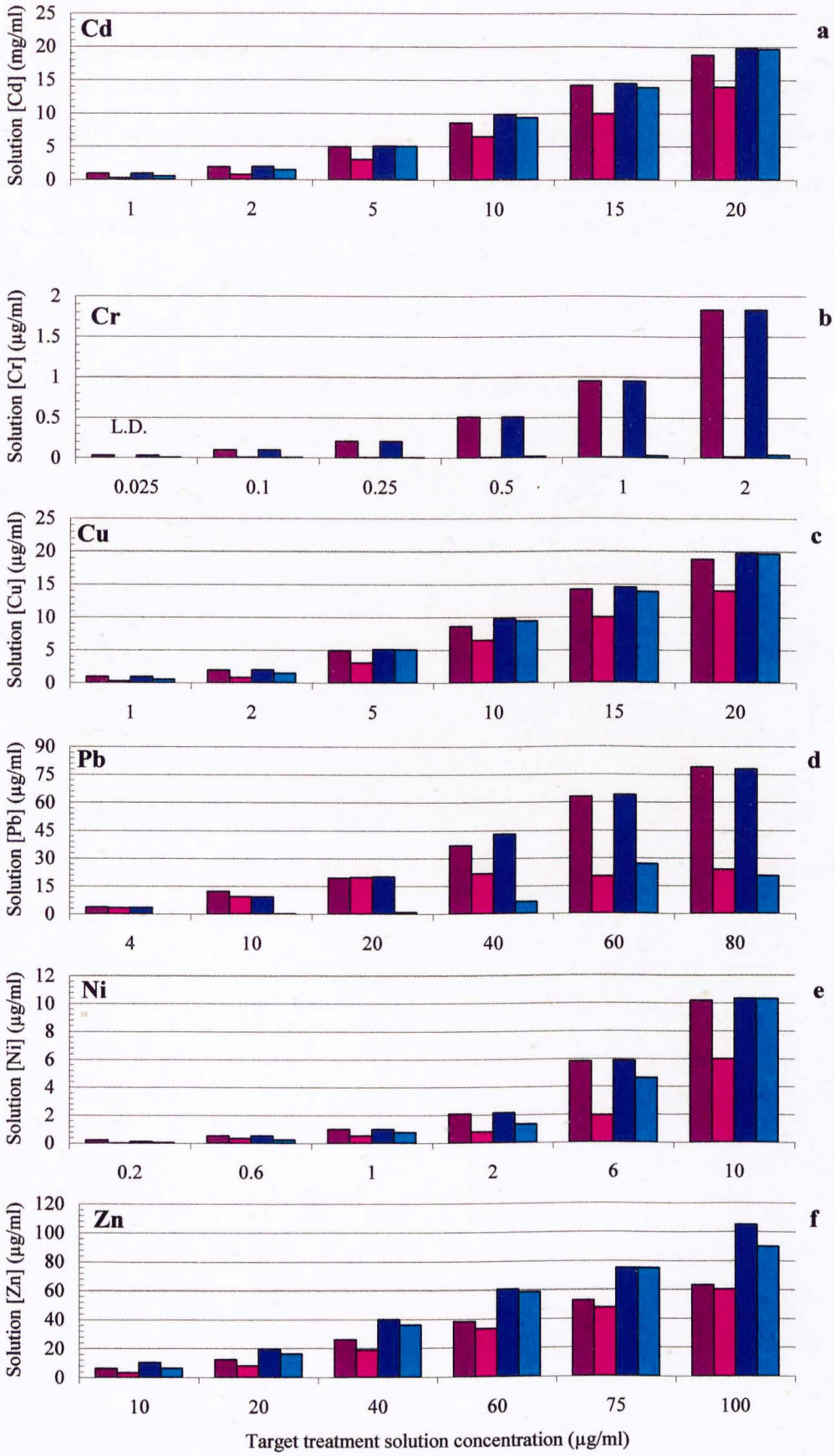

Figure 5.2 NFT metal solution concentrations. Values represent the actual solution concentrations in the newly prepared solutions on: day 0 (■) and day 7 (■) and the concentrations of the same solutions after 7-d plant growth: day 7 (■) and day 14 (■), respectively. Individual histograms represent (a) Cd, (b) Cr, (c) Cu, (d) Pb, (e) Ni and (f) Zn. Target concentrations are detailed in Table 2.10 (Section 2.8.3).

5.1.5 Growth response of flax to the NFT solutions

The plant growth response was determined by a qualitative assessment of the plants grown in the NFT solutions compared to plants grown in control solutions. Plants were assessed based on their appearance at the end of the 14-d treatment period and assigned a score of 0–5. The criteria used to assess the plants and the associated scores given are detailed in Table 5.1.

Table 5.1 Plant growth response scores and criteria

Score	*Plant condition criteria with respect to control plants.*
5	Plants at least as healthy as control plants.
4	Plants showing mild chlorosis and/or a small reduction in shoot and/or root length.
3	Plants showing more pronounced chlorosis and reduction in shoot and/or root length.
2	Plants severely chlorotic with either browning or loss of leaves and/or pronounced stunting or browning of roots.
1	Plants severely chlorotic with many leaves lost and/or some dead plants present. No root growth observed during the 14-d treatment period.
0	Fewer than 10% of plants alive and surviving plants condition as score 1.

The plants grown in each solution in the NFT system had a uniform growth response within each of the troughs.

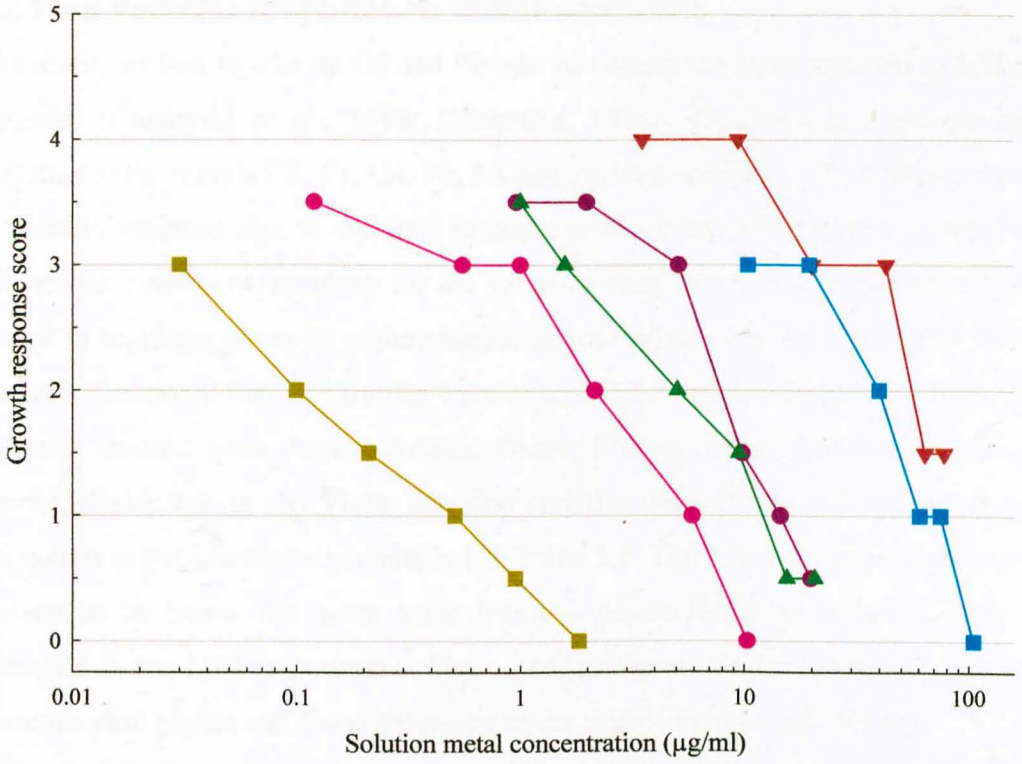

Figure 5.3 Flax growth response to NFT solution concentrations. Plots represent the plant growth response score for Cd (-●-), Cr (-■-), Cu (-▲-), Pb (-▼-), Ni (-●-) and Zn (-■-). Plant growth response criteria for each score are detailed in Table 5.1.

Three metals (Cr, Ni and Zn) were lethally toxic to > 90% of the flax plants grown at their highest solution concentration (Fig. 5.3). Many dead plants were observed at the highest Cd and Cu NFT solution concentrations, although fewer than 90%, generating a growth response score of 0.5. The plants grown in the Pb solutions were the only plants to score greater than 1 at the highest NFT solution concentration. Chromium was the most toxic metal to the flax plants as it was lethal at a concentration of 2 µg/ml. Plants grown in the Pb and Zn solutions were exposed to the highest solution concentrations, 80 and 100 µg/ml, respectively and Pb was found to be less toxic than Zn as the plants grown in the 80 µg/ml Pb solution had a better growth response than the plants grown in the 75 µg/ml Zn solution (Fig. 5.2). Thus, Pb was the least toxic metal to flax plants in the NFT study. The metals can be ranked in order of observed decreasing toxicity as follows:

$$Cr > Ni > Cu \geq Cd > Zn > Pb$$

5.1.6 NFT summary

The NFT study revealed that, of the six metals in the study, Cr was the most toxic to flax. Lead was observed not only to be the least toxic metal to flax but also to be removed in the greatest quantity from solution. The NFT study provided a valuable guide to the toxicity of the metals in solution indicating their toxicity independent of matrix interferences.

5.2 Flax varietal response to metal solutions

The ability of flax to take up Cd and Pb into its tissues has been reported to differ between varieties (Cieslinski *et al.*, 1996; Lukipudis, 1994). The growth responses of 12 flax varieties to the metals Cd, Cr, Cu, Pb, Ni and Zn were studied, over a 28-d growth period, to identify varieties able to maintain biomass productivity when grown in metal solutions. The tissue metal concentrations for the varieties were determined at the end of the growth period to highlight potential phytoremediation candidates. From a total of 24 flax varieties on the UK national list, 12 varieties were available through kind provision by NIAB. These varieties studied were Argos, Ariane, Diane, Electra, Elise, Escalina, Evelin, Hermes, Martta, Rasia, Viking and Viola. The flax varieties were grown in solutions of each of the six metals at the concentration detailed in Table 5.2. The solution concentrations used were chosen to be below the acute toxic levels – where death or severe growth problems occurred in the NFT experiment. These concentrations were chosen in order to identify varieties with higher and lower tolerance to the metals than variety Viking.

Table 5.2 Metal solution concentrations used in flax varietal comparisons. The colours used to represent the elements in Fig. 5.4–5.16 are also shown.

Element	Concentration (µg/ml)	Graph colour
Cadmium	5.0	
Chromium	1.0	
Copper	2.0	
Lead	40.0	
Nickel	1.0	
Zinc	10.0	
Control	-	

5.2.1 Flax plant growth response to metal solutions.

In the screening experiment the flax plants were germinated and grown in growth collars (Section 2.8.2). The growth collars were placed in seed trays with 12 collars per tray, with one collar for each variety (Section 2.9). As the metal solutions were added to the seed trays, this ensured that the roots of each of the 12 varieties shared the same solution.

5.2.1.1 Germination and survival

The number of flax plants recorded for each variety–metal solution system (Fig. 5.4) was a measure of the number of plants able to germinate and survive until the end of the growth period.

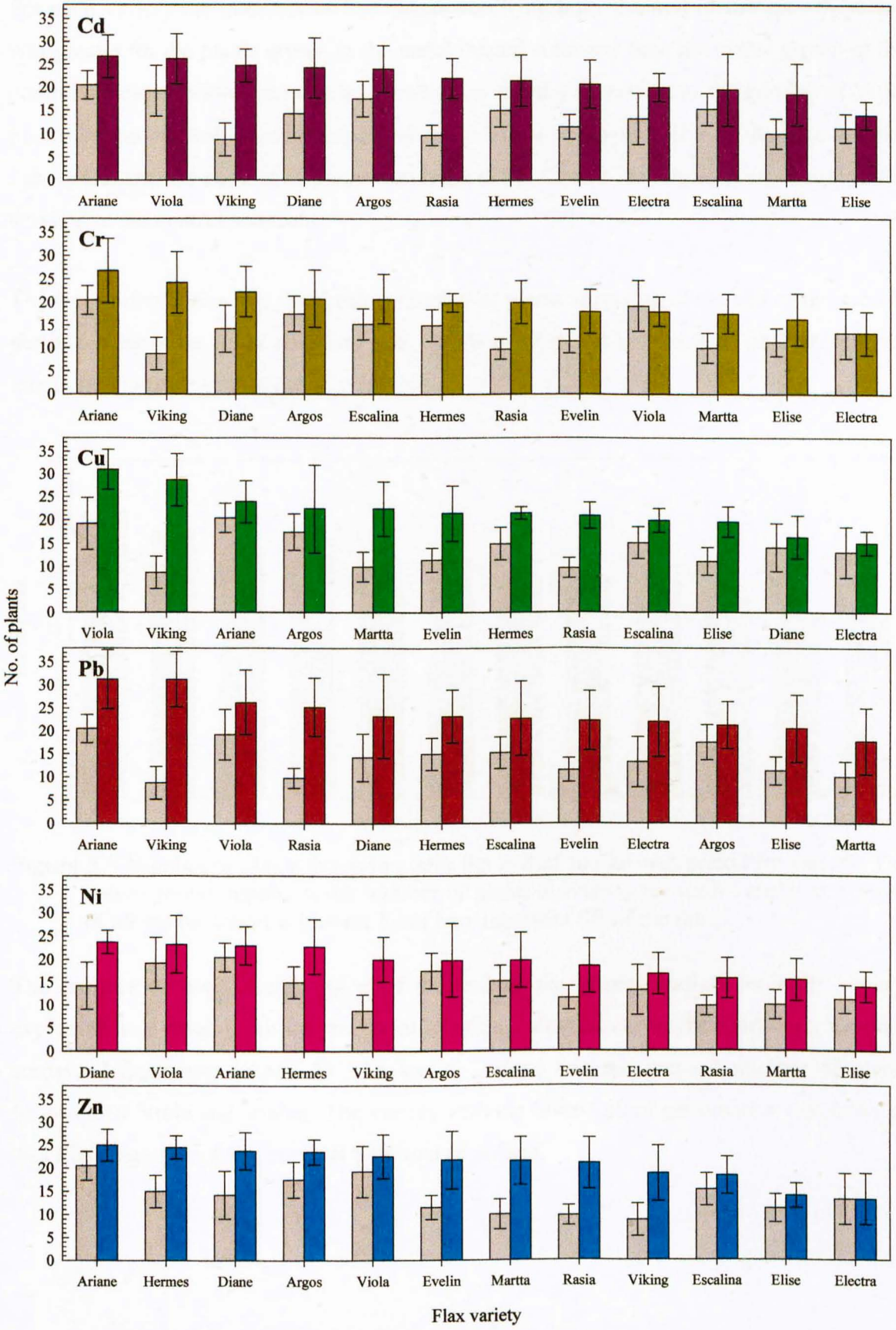

Figure 5.4 Mean number of plants per flax–metal system. Values shown are the mean number of plants for each flax variety, per collar, grown in: Cd (■), Cr (■), Cu (■), Pb (■), Ni (■), Zn (■) and control (■) solutions. The control treatments for each variety are shown with each metal treatment to allow comparison. Error bars represent SE of the mean.

For each variety the numbers of flax plants surviving until the end of the growth period was greater for the plants grown in the metal treated solutions than the plants grown in the control solutions. Viola grown in the Cr solution was the exception, as the number of Viola plants in the control solution exceeded those in the Cr solution. Additionally, for the variety Electra, the number of plants surviving in the Cr and Zn solutions was equal to the survival in the control solutions.

The flax variety Arian had the greatest number of plants surviving at the end of the growth period in four of the metal solutions (Cd, Cr, Pb and Zn) and had the third greatest survival rate in the remaining solutions (Cu and Ni).

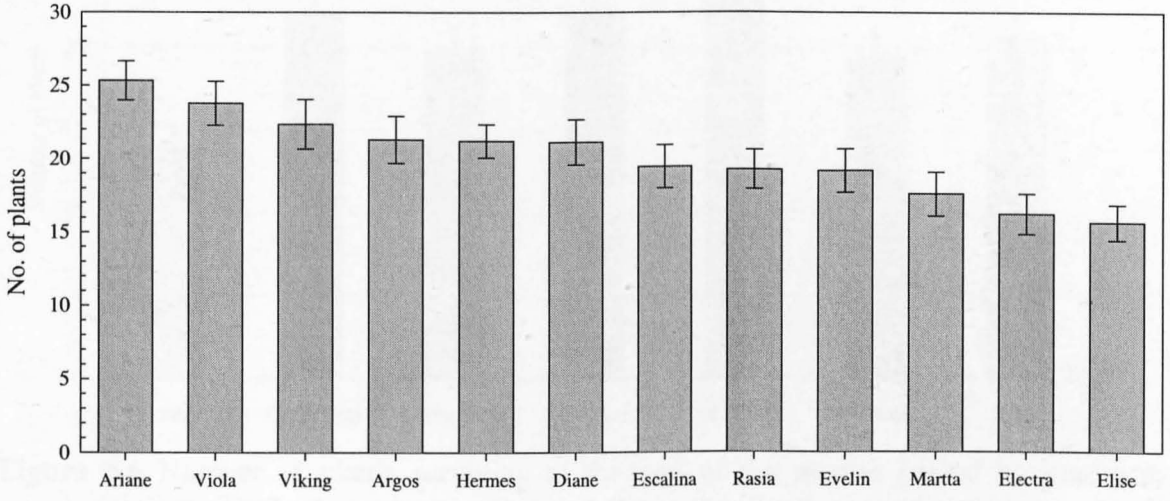

Figure 5.5 Number of plants surviving until the end of the growth period by variety. The values shown represent the number of plants surviving for each variety as a mean of all the treatment solutions. Error bars represent SE of the mean.

The number of plants surviving until the end of the growth period for each variety, expressed as a mean of all the treatment solutions, allowed comparison between varieties across all treatments (Fig. 5.5). The variety Ariane had the greatest number of plants followed by Viola and Viking. The variety with the lowest plant germination and survival was Elise, equivalent to a survival 64% that of Ariane.

The number of plants surviving until the end of the growth period for each treatment solution, expressed as a mean of all the varieties, allowed comparison between treatment solutions across all varieties (Fig. 5.6). For all six metal treatments, the mean flax plant germination and survival at the end of the growth period was greater than in the control solution. The increase in germination and survival from the control solution to the metal treated solutions may be due to the increased ionic strengths of the metal solutions. Lead had the highest value for germination and survival followed by Cu and Cd.

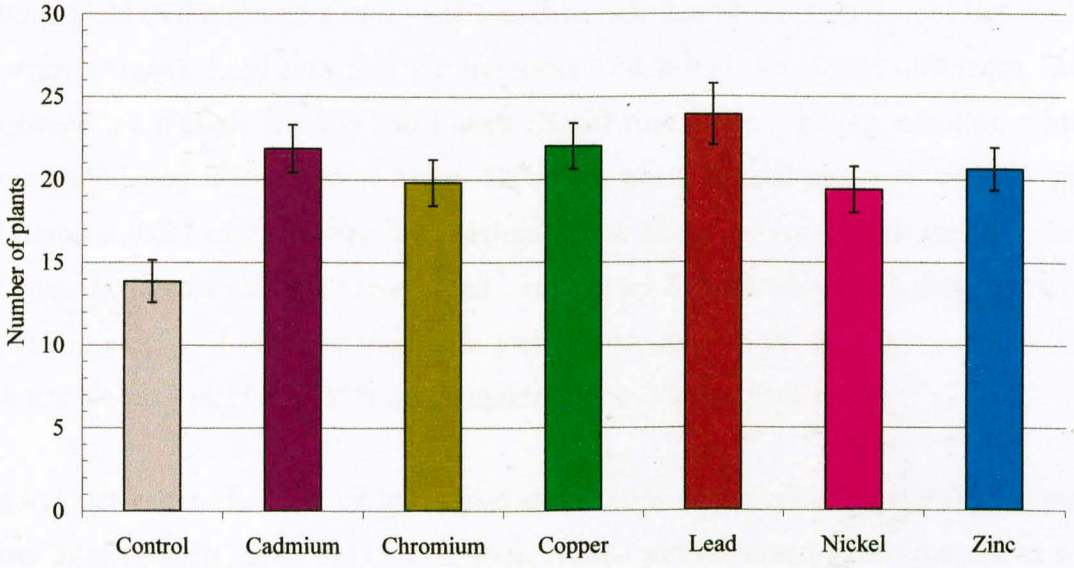

Figure 5.6 Number of plants surviving at the end of the growth period by treatment solution. The values shown represent the mean shoot survival number over all varieties for the Cd (■), Cr (■), Cu (■), Pb (■), Ni (■), Zn (■) and control (■) treatments. Error bars represent SE of the mean. All of the values for the metal treatments were significantly different from the control value (*t* test, $P<0.05$). Comparisons were not made directly between metals which were not at equimolar concentrations.

5.2.1.2 Root dry weight

Dry weight of root tissue indicated the impact of the metal solutions on the plants ability to produce root biomass. In order to compensate for the variation in survival, the dry weight of root tissue was expressed as the mean weight per plant (Fig. 5.7).

For each of the metals studied, the mean dry weight of roots showed a gradual decline in root tissue weight from the varieties with the highest to the lowest root weights (Fig. 5.7). Lead was the exception to this decline, where the variety with the second highest root tissue yield (Viking) had a root yield less than half that of the highest root tissue yielding variety (Argos). Lead was also the treatment which had the largest difference (2.8 mg) between the highest (Argos) and lowest (Elise) root tissue yielding varieties, with Elise root yield being < 20% that of Argos. Cadmium was the metal treatment with the smallest difference (0.87 mg) between the maximum and minimum root tissue yielding varieties; Argos, the lowest yielding variety, had a root tissue concentration 62% that of the highest yielding variety, Rasia. The minimum yielding varieties in the remaining metals (Cr, Cu, Ni and Zn) had yields 42–47% of the maximum yielding varieties.

Individual varieties could not be singled out as consistently yielding higher root material, over all the metals, as all but two varieties, Ariane and Hermes, yielded root tissue weights within the top three values for one or more of the metals (Fig. 5.7). The highest root tissue yielding variety for each metal was Rasia (Cd), Electra (Cr), Argos (Cu and Pb) Elise (Ni), and Viking (Zn) (Fig. 5.7). This indicated that optimising varietal root metal tolerance must be done on an element by element basis.

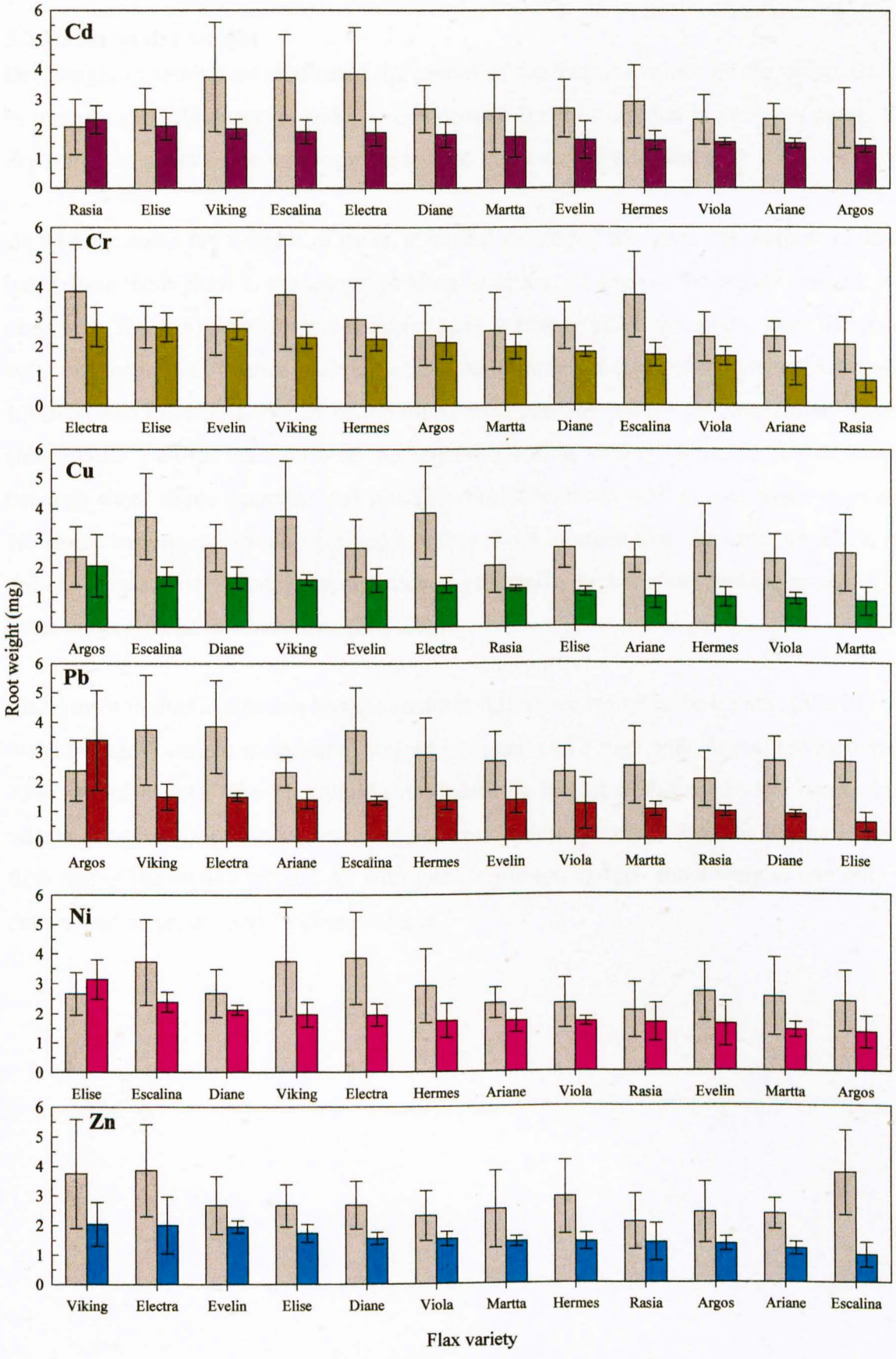

Figure 5.7 Mean dry weight of flax plant root tissue. Values shown are the mean dry weight (mg/plant) of root tissue per plant. These values were calculated by dividing the total tissue weight per growth collar by the number of plants in that collar, before a mean from the four replicate growth collars was obtained. Other details as in Fig. 5.4.

5.2.1.3 Shoot dry weight

Dry weight of shoot tissue indicated the impact of the metal solutions on the plants ability to produce shoot biomass. In order to compensate for the variation in survival, again, the dry weight of shoot tissue was expressed as the mean weight per plant (Fig. 5.8).

As with the mean dry weights of roots, a gradual decline in the mean dry weights of shoot tissue from the highest to the lowest yielding varieties, for each of the metals studied, was observed (Fig. 5.8). The control solution gave a higher yield, per plant, than the metal solutions for all the varieties, with the exception of three variety–metal systems (Argos–Cr, Elise–Ni and Elise–Zn). For all of the metal solutions, the lowest yielding variety gave a shoot tissue yield at least 60% of the highest yielding variety, therefore the difference between shoot tissue response was less than the differences seen in root tissue response. No single variety consistently yielded a higher shoot biomass than the other varieties, nor did any single variety–metal system produce a markedly higher shoot yield than any of the other variety–metal combinations (Fig 5.7).

Flax grown in the Cd solution had the smallest difference between the highest (Martta) and lowest (Argos) variety mean shoot weight per plant (4.73 mg) with Argos having a yield 73% that of Martta. The Zn solution produced the largest difference in the mean shoot weight per plant from the highest to the lowest yielding variety, with the lowest (Argos) 62% that of the highest (Elise). As with roots, optimum varietal shoot weights can only be determined on an element by element basis.

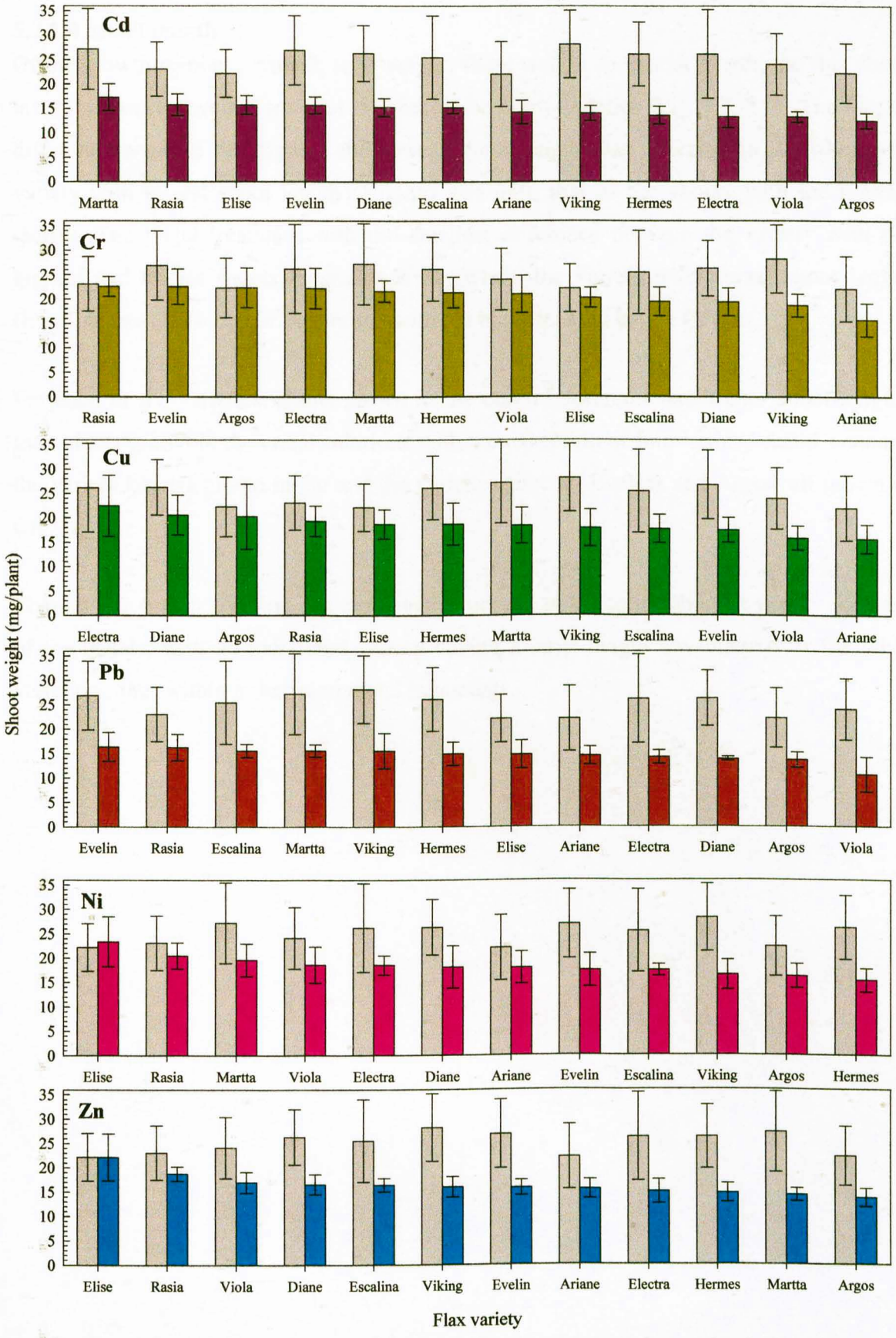

Figure 5.8 Mean dry weight of flax plant shoot tissue. Values shown are the mean dry weight (mg/plant) of shoot tissue per plant. Other details as Fig. 5.7.

5.2.1.4 Shoot length

Of the growth response criteria: root weight, shoot weight and shoot length, the flax shoot length response gave the smallest differences between varieties (Fig. 5.7–5.9). The largest difference between the highest and lowest shoot length was observed in Zn where the variety with lowest shoot length (Viking) was 68% that of the variety with the highest (Elise). The metal treatment with the smallest difference between the variety with the highest and lowest shoots lengths was Pb, where the variety with lowest shoot length (Escalina) was 80% that of the variety with the highest shoot length (Martta).

For each of the varieties, plants grown in the control solutions had higher shoot lengths than plants grown in the metal solutions with the exception of four variety–metal systems: the variety Electra grown in Cu and the varieties Electra, Eveline and Argos, all grown in Cr.

There was a gradual decline in shoot length from the tallest to the shortest variety for each of the metals, with no individual variety having a large height advantage over the other varieties either within or between metal treatments.

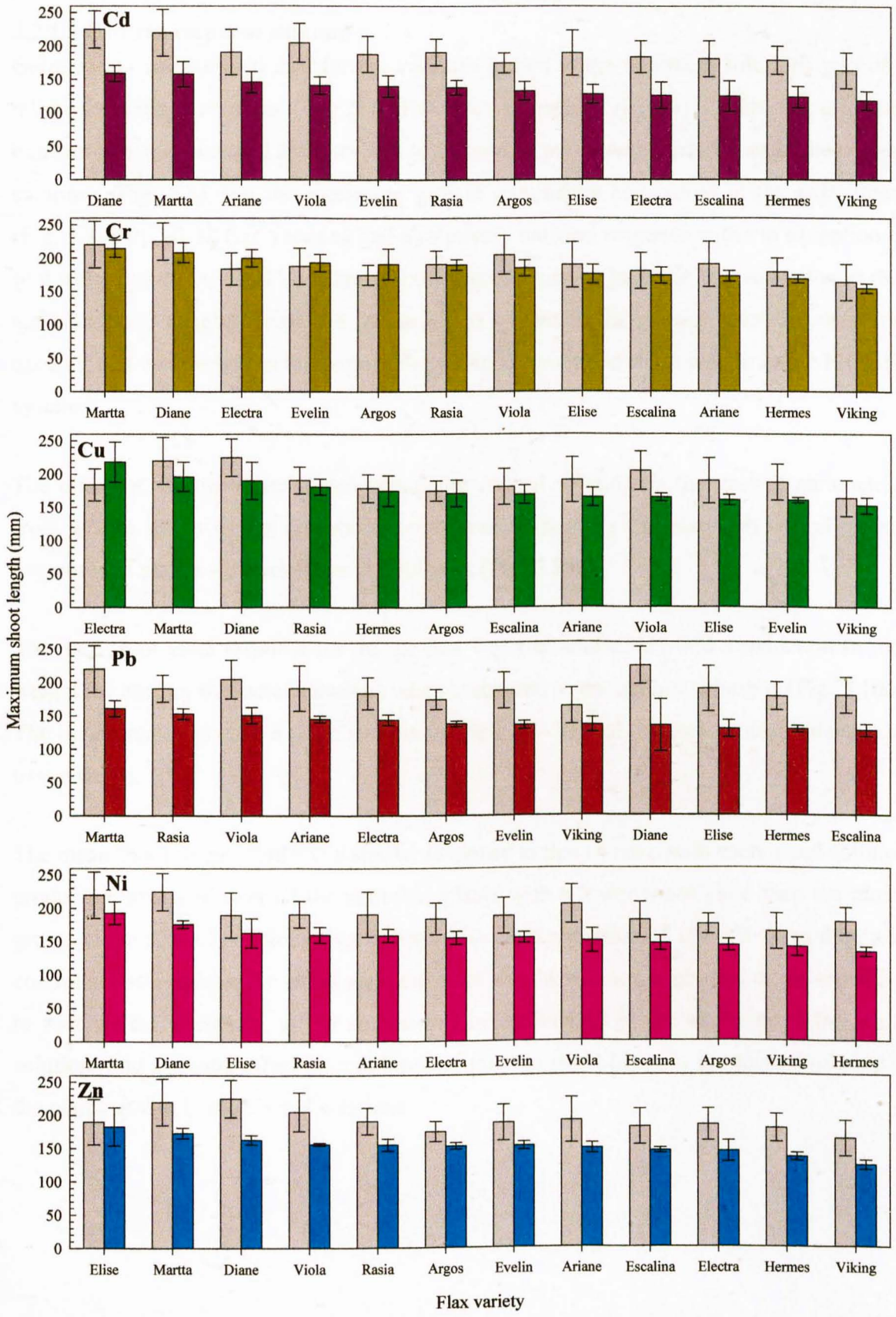

Figure 5.9 Mean shoot length of flax plants. Values shown are the mean of the maximum shoot lengths for each growth collar. Other details as in Fig. 5.4.

5.2.1.5 Growth response summary

Germination and survival data for the varieties grown in the treatment solutions was of a relatively uniform response (Fig. 5.4). However, in each of the metal solutions, a greater number of plants emerged and survived to the end of the growth period than in the control solutions (Fig. 5.5). For the remaining growth parameters considered in the experiment (Fig. 5.7–5.9), all 12 flax varieties had a relatively uniform response with the exception of root weight in the Argos–Pb system. All the metal solutions gave rise to a reduction in root and shoot dry weights compared to the plants grown in the control solutions, with the exception of root weight in the Argos–Pb system and root and shoot weight in the Elise–Ni system.

The effect of the metal treatments versus the control solution on the growth parameters: (root weight, shoot weight and shoot length) can be seen by expressing the mean growth responses of all the varieties for each treatment (Fig. 5.10).

The mean root yield response for the 12 flax varieties studied showed a reduction in root weight for each of the metal solutions when compared to the control solutions (Fig. 5.10a). The metal treatments had a mean root tissue yield 48–70% of the mean control plants root tissue yields.

The mean shoot tissue yield had a similar response to that of roots with each metal solution producing, averaged over all the varieties, plants with a lower shoot yield than the plants grown in the control solutions (Fig. 5.10b). The yield reduction of 18–41% from the mean control shoot weight to the metal solution shoot weight was not as marked as the reduction in root yields. Therefore, at the concentrations considered in the experiment, the metal solutions had a greater effect on root biomass than on shoot biomass production relative to the plants grown in the control solutions.

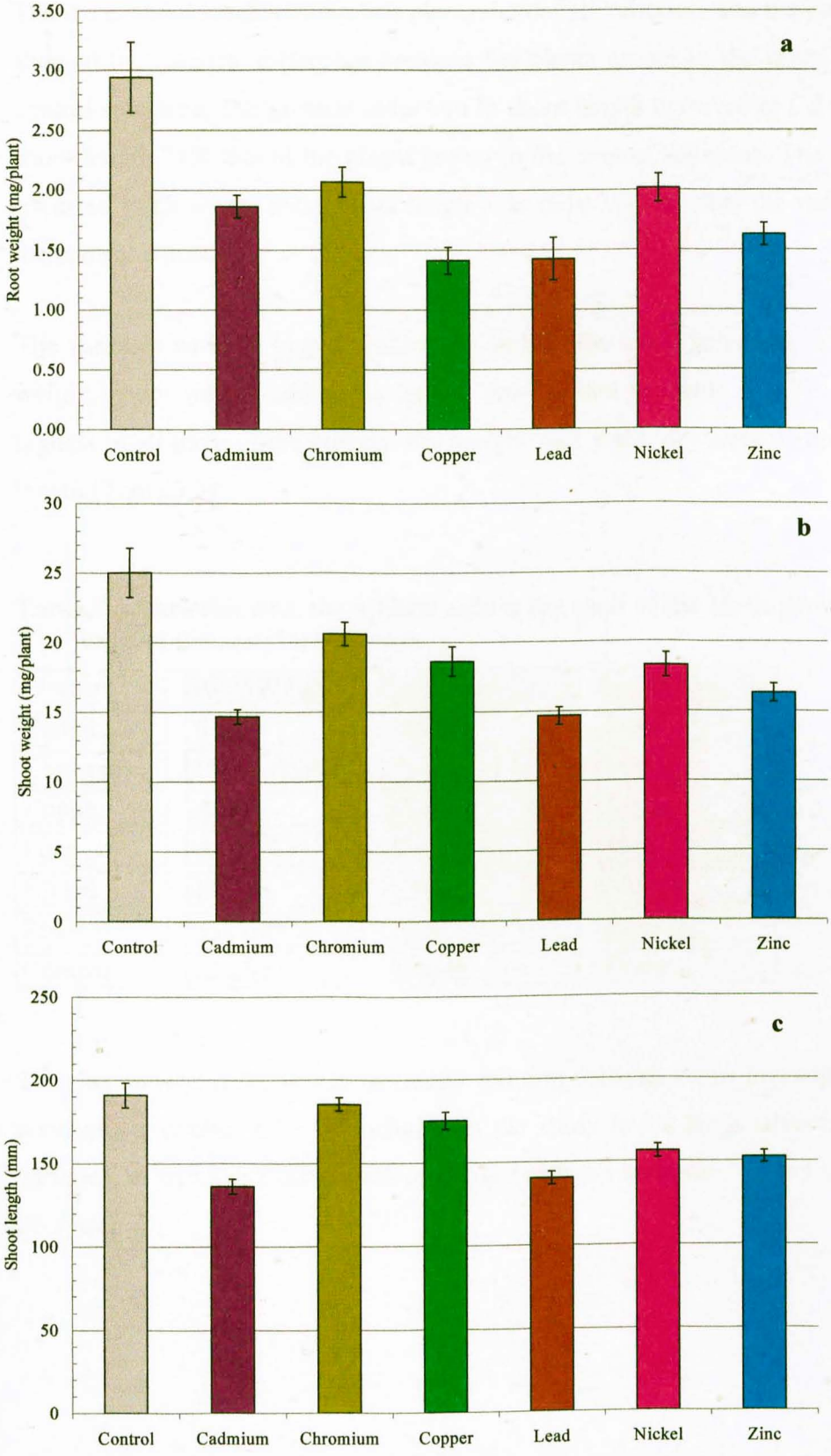

Figure 5.10 Mean dry weights of flax roots, shoots and shoot length in each of the metal solutions. Values shown are means of (a) root dry weight (mg/plant), (b) shoot dry weight (mg/plant) and (c) maximum shoot length (mm). Means are the mean of each growth response criteria, per plant, over all varieties grown in each metal treatment. Error bars represent SE of the mean . Shoot length of plants grown in the Cr solution was the only value which was not significantly different from the control values (*t* test, $P<0.05$). Comparisons were not made directly between metals which were not at equimolar concentrations.

The mean shoot lengths of the flax plants, across all varieties, was the growth criterion that showed the smallest difference between the plants grown in the metal solutions and the control solutions. The greatest reduction in shoot length occurred in Cd which had a mean shoot length 71% that of the plants grown in the control solutions. The smallest reduction occurred in Cr whose mean shoot length was only 3% less than the mean shoot length of the control plants.

The varieties with the highest values for each of the metal solutions, as measured by root weight, shoot weight and shoot length, are detailed in Table 5.3. No one variety was highest in all three of the criteria: dry weight root yield, dry weight shoot yield and shoot length (Table 5.3).

Table 5.3 Varieties with the highest values for each of the three growth parameters in each of the metal treatments

Element	Root Weight	Shoot weight	Shoot Length
Cadmium	Rasia	Martta	Diane
Chromium	Electra/Elise	Rasia	Martta
Copper	Argos	Electra	Electra
Lead	Argos	Evelin	Martta
Nickel	Elise	Elise	Martta
Zinc	Viking	Elise	Elise
Control	Electra	Viking	Diane

The flax varietal responses to the metal solution concentrations investigated, revealed that none of the twelve varieties included in the study had a large advantage over the other varieties, in terms of their growth response (with the exception of root yield in the Argos–Pb system).

5.2.2 Metal uptake into flax tissues

Flax plant shoot and root tissue concentrations for Cd, Cr, Cu, Pb, Ni and Zn were calculated for each of the twelve varieties. Due to the small weight of root tissue yielded by the plants it was not possible to replicate the root analyses (Section 2.9). Values have nevertheless been shown to give an indication of root concentration and are discussed.

Individual root and shoot tissue metal concentrations were considered (Fig. 5.11–5.16) and the highest yielding varieties with their corresponding metal uptake values, for each metal, are summarised at the end of the section (Table 5.4). Data for the mean uptake over all flax varieties are also presented (Table 5.5).

Cadmium

Viola was the variety with the highest shoot tissue Cd concentration, 191 μg/g, which was significantly more than the shoot Cd concentration of variety Martta, the next most concentrated shoot tissue (Fig. 5.11a; Table 5.4). The variety with the lowest Cd shoot tissue concentration was Escalina (118 μg/g) which had a shoot Cd concentration 62% that of Viola. The shoot Cd concentration of the flax varieties was 24–38 times the solution Cd concentration, representing a phytoaccumulation of Cd. The mean phytoaccumulation of Cd into flax shoot tissue over all twelve varieties was 30 times the solution concentration (Table 5.5).

In addition to having the highest shoot tissue Cd concentration Viola was also the variety which had the highest root tissue Cd concentration at 3051 μg/g (Fig. 5.11b; Table 5.4). The lowest root tissue Cd concentration observed was for variety Elise at 1930 μg/g, representing 63% of the maximum recorded root tissue concentration. The mean root concentration, over all varieties, was ~500 times the Cd solution concentration and correspondingly, as a mean over all varieties, root Cd uptake was ~17 times greater than shoot uptake (Table 5.5).

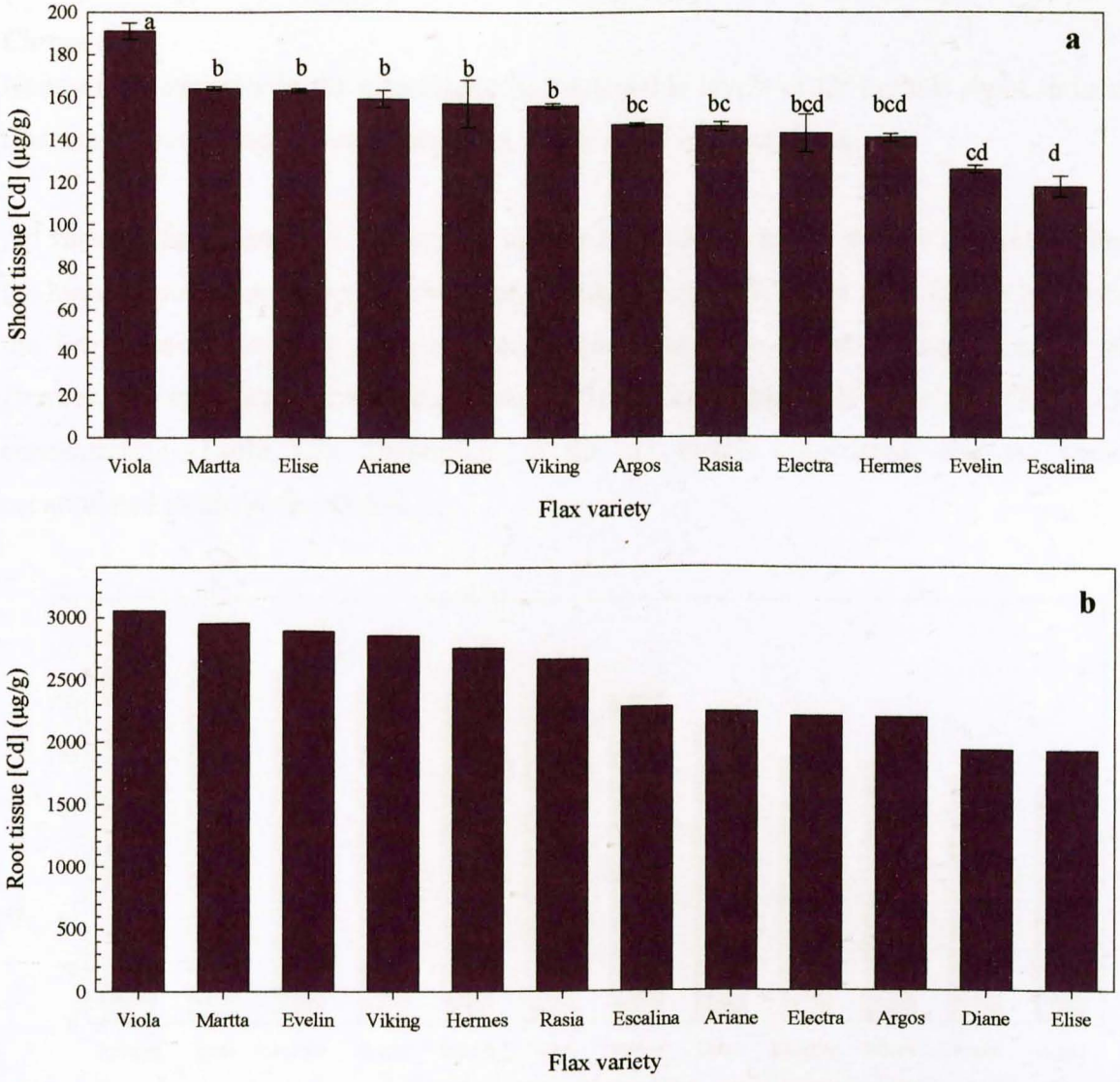

Figure 5.11 Flax tissue Cd concentration. Values shown represent: (a) mean shoot tissue Cd concentration (µg/g) and (b) pooled root tissue Cd concentration (µg/g), for each variety (Section 2.9). Error bars represent SE of the mean. In (a) the values are significantly different, ANOVA. Values sharing the same letter are not significantly different, *t* test with Bonferroni correction, $P>0.05$.

Chromium

None of the varieties in the experiment had detectable levels of Cr in their shoot tissues. Chromium was the only metal which was not detected in shoot tissue.

All varieties had detectable levels of Cr in their root tissue with the variety Hermes having the highest root tissue Cr concentration at 148 μg/g (Fig. 5.12; Table 5.4). The variety with the lowest root tissue Cr concentration (Argos) had 61% of the Cr concentration of Hermes. The mean root accumulation over all flax varieties was ~120 times the solution Cr concentration (Table 5.5). Chromium, of the six metals considered, was the metal accumulated to the lowest extent.

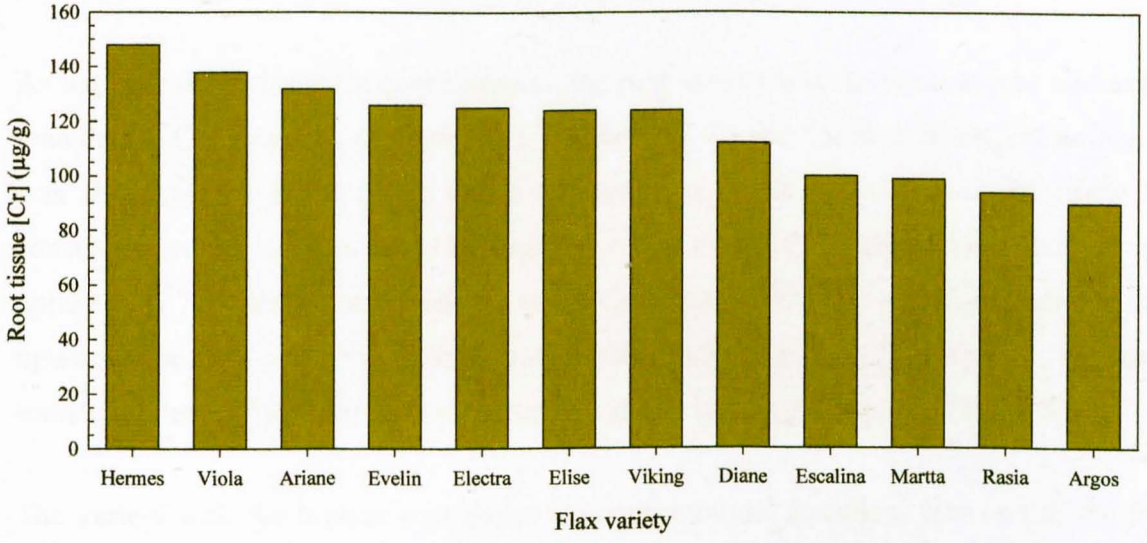

Figure 5.12 Flax tissue Cr concentration. Values shown represent the pooled root tissue Cr concentration (μg/g), for each variety (Section 2.9).

Copper

The highest shoot tissue Cu concentration was eight times lower than that of Cd, however, like Cd, the variety Viola had the highest shoot tissue Cu concentration (23 μg/g; Table 5.4). Copper was, with the exception of Cr, the metal taken up at the lowest shoot tissue metal concentration. The variety with the second highest shoot tissue Cu concentration was Viking at 21 μg/g. The remaining varieties all had an uptake < 20 μg/g (Fig 5.13a). The lowest Cu uptake by shoots (14 μg/g) was shared by four varieties: Electra, Elise, Escalina and Rasia and accounted for an uptake 60% that of Viola. The mean shoot tissue Cu concentration (17 μg/g), over all varieties, represented a Cu accumulation by flax shoots eight times that of the solution concentration (Table 5.5). None of the shoot tissue Cu concentrations were significantly different from one another (Fig. 5.13a).

As with the shoot tissue Cu concentration, the root tissue Cu concentration was also lower than that of Cd, however, the difference between the Cd and Cu root tissue concentrations was less than for shoot tissue concentrations (Fig. 5.13b). The mean root tissue Cu concentration over all varieties (1196 μg/g) was half that of Cd (Table 5.5). This mean root uptake was 72 times greater than the shoot Cu uptake and was the largest ratio of root uptake:shoot uptake observed for all the metals studied, making Cu, after Cr, the metal transferred least efficiently from root tissue to shoot tissue by flax plants (Table 5.5).

The variety with the highest root tissue Cu concentration, Escalina, was one of the four varieties with the lowest shoot tissue Cu concentration. Escalina had a root tissue concentration of 1537 μg/g which was an accumulation of 768 times the solution concentration. The lowest root tissue Cu concentration was observed in the variety Argos at 1068 μg/g, which was 69% of the root tissue Cu concentration of Escalina.

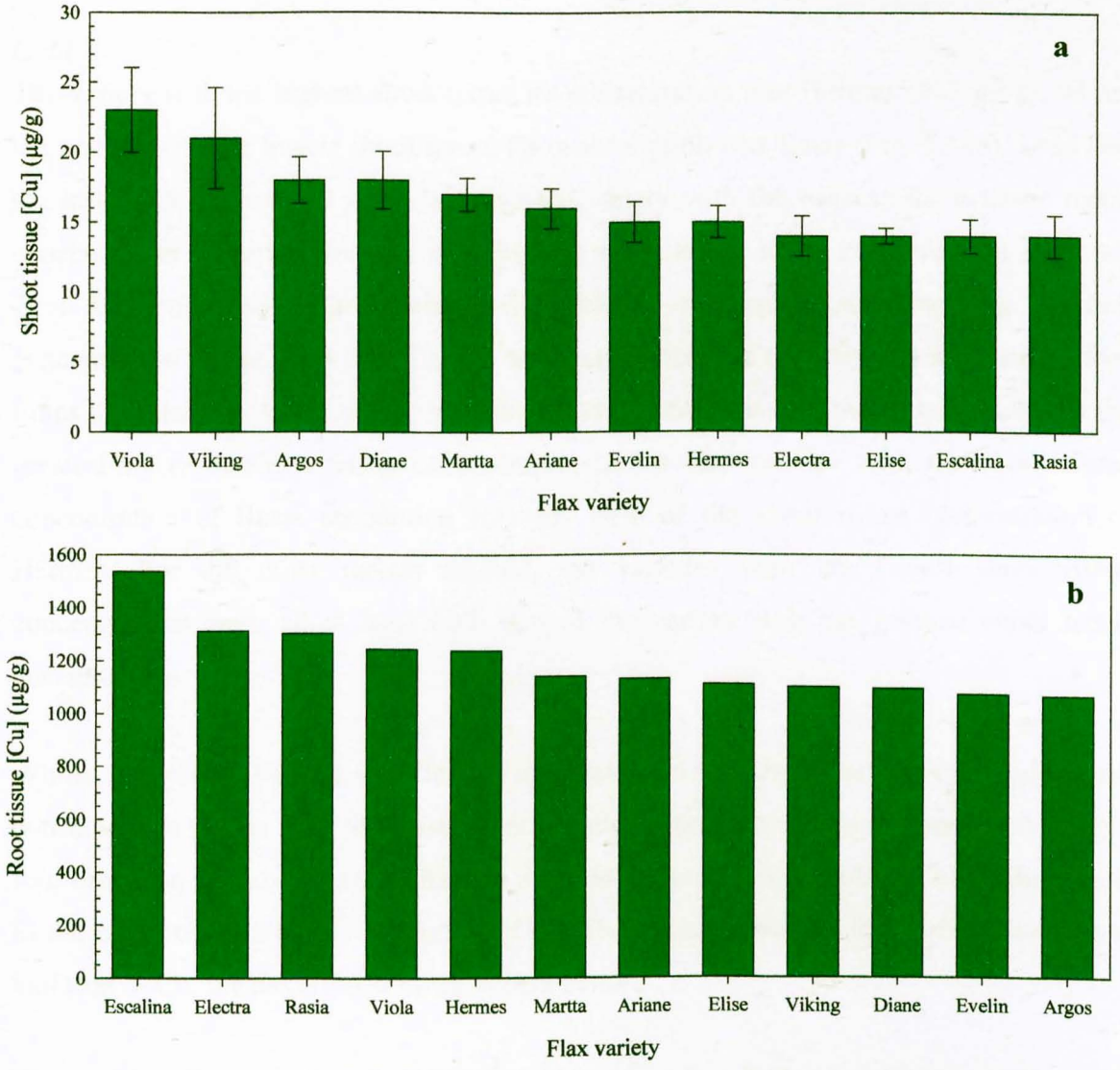

Figure 5.13 Flax tissue Cu concentration. Values shown represent: (a) mean shoot tissue Cu concentration (µg/g) and (b) pooled root tissue Cu concentration (µg/g), for each variety (Section 2.9). Error bars represent SE of the mean. Using *t* test with Bonferroni correction, shoot tissue concentrations were not significantly different ($P<0.05$).

Lead

The variety with the highest shoot tissue Pb concentration was Hermes (243 μg/g), whilst the variety with the lowest shoot tissue Pb concentration was Rasia (Fig. 5.14a). Lead had the largest difference (80 μg/g) between the variety with the highest shoot tissue metal concentration (Hermes) and the next highest shoot tissue metal concentration (Martta). This difference meant that Hermes was able to accumulate six times the solution concentration in the plant shoot tissue whereas Martta was only able to accumulate four times the solution concentration in its shoot tissue. Lead was also the metal which had the greatest range in shoot tissue concentration across the varieties with the shoot tissue concentration of Rasia accounting for only 44% of the shoot tissue concentration of Hermes. For the other metals studied, the varieties with the lowest shoot tissue concentrations were all at least 60% that of the variety with the greatest shoot tissue concentration.

Whilst, as a mean of all the varieties, Pb was present in flax shoot tissue at the third highest concentration (Table 5.5), this tissue concentration represented a Pb accumulation of only four times the solution Pb concentration and was the lowest accumulation of all the metals in the experiment, with the exception of Cr. The accumulation of Pb in shoot tissue was half that of Cu, the next lowest metal accumulation.

The varieties Elise and Viola had the highest root tissue Pb concentration with tissue concentrations of ~9500 μg/g (Fig 5.13b; Table 5.4). Argos was the variety with the lowest root tissue Pb concentration (4505 μg/g) which was an uptake of less than half that of Elise. Lead was present in root tissue at a greater concentration than any of the other metals studied; the mean root tissue Pb concentration over all varieties was 6719 μg/g which was more than double the root tissue Zn concentration, the next most accumulated metal (Table 5.5). The high root tissue Pb loading was, however, offset by the high solution Pb loading, therefore the accumulation of Pb by roots in proportion to the solution concentration was lower than the other metals, with the exception of Cr (Table 5.5).

Flax, as a mean of all the varieties, had 46 times more Pb in the roots than shoots suggesting Pb, like Cr and Cu, was not readily translocated from roots to shoots (Table 5.5).

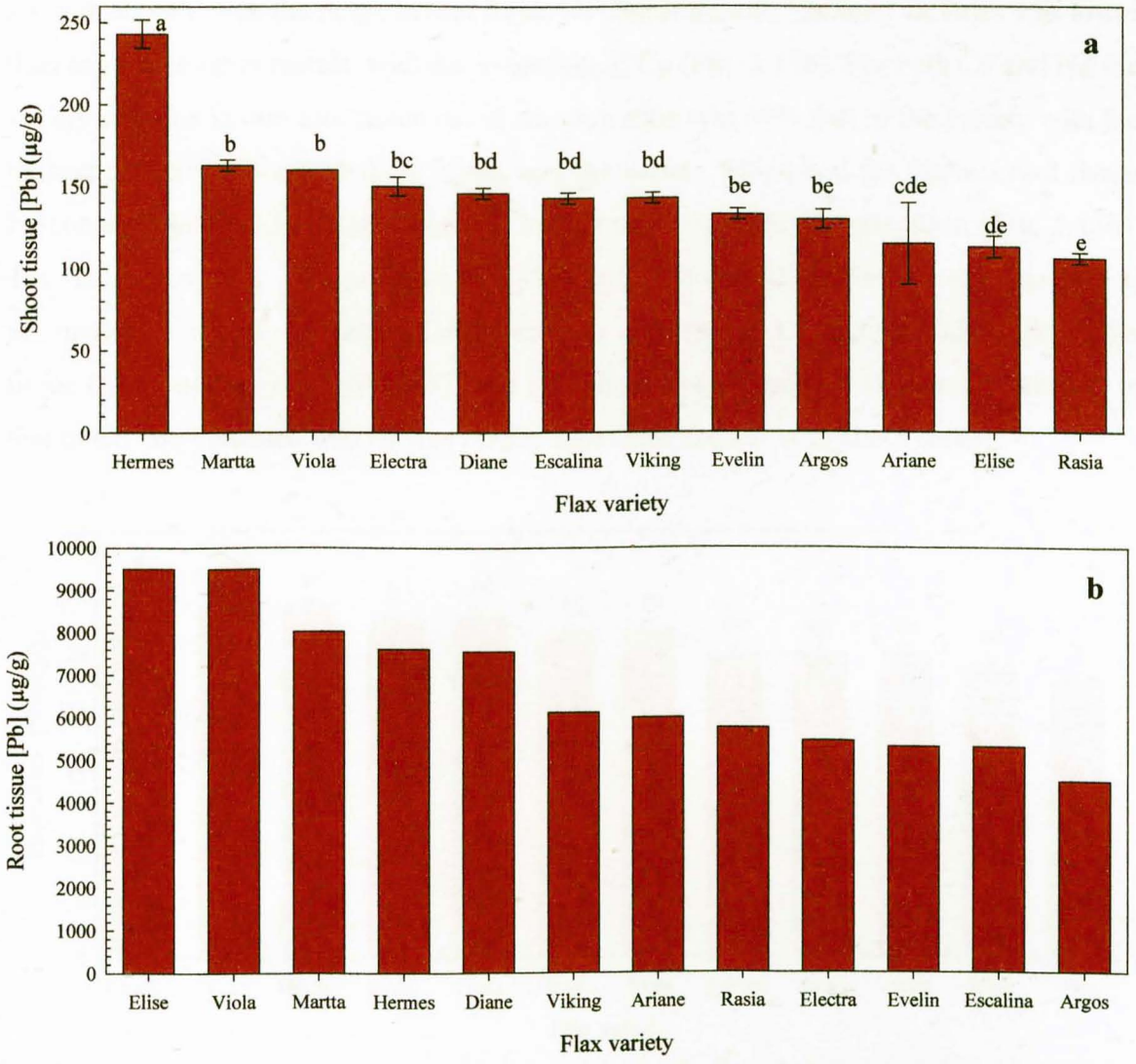

Figure 5.14 Flax tissue Pb concentration. Values shown represent: (a) mean shoot tissue Pb concentration (µg/g) and (b) pooled root tissue Pb concentration (µg/g), for each variety (Section 2.9). Error bars represent SE of the mean. In (a) the values are significantly different, ANOVA. Values sharing the same letter are not significantly different, *t* test with Bonferroni correction, $P>0.05$.

Nickel

The variety Escalina had the highest shoot tissue Ni concentration at 31 µg/g but this treatment was not significantly different from the next six highest Ni accumulating varieties. The variety Diane had the lowest shoot tissue Ni concentration at 23 µg/g (Fig 5.14a). Of all the metals, Ni exhibited the narrowest range of shoot tissue concentrations between the varieties, with Diane having a shoot tissue concentration 75% that of Escalina. Ni exhibited the lowest variation in metal uptake across the 12 flax varieties.

Nickel was present in shoot tissue at 23–31 times the solution Ni concentration, with a mean across all varieties of 27 times the solution Ni concentration, which, was an accumulation similar to that of Cd, but > three times that of Cu.

As with shoot tissue, the range in root tissue Ni concentrations between varieties was lower than any of the other metals, with the exception of Cu (Fig. 5.15b). For both Cu and Ni, the variety with the lowest root tissue metal concentration was 69% that of the variety with the highest root tissue concentration. Viola was the variety which had the highest root tissue Ni concentration whilst Ariane had the lowest root tissue Ni concentration (Fig. 5.15b). The mean root Ni concentration of 479 µg/g, across all varieties, represented an accumulation similar to that of Cd, albeit at a concentration five times lower than root tissue Cd concentration (Table 5.5). The root:shoot uptake ratio of 18 was also similar to that of Cd and indicated that Ni was readily mobilised from root to shoot tissue.

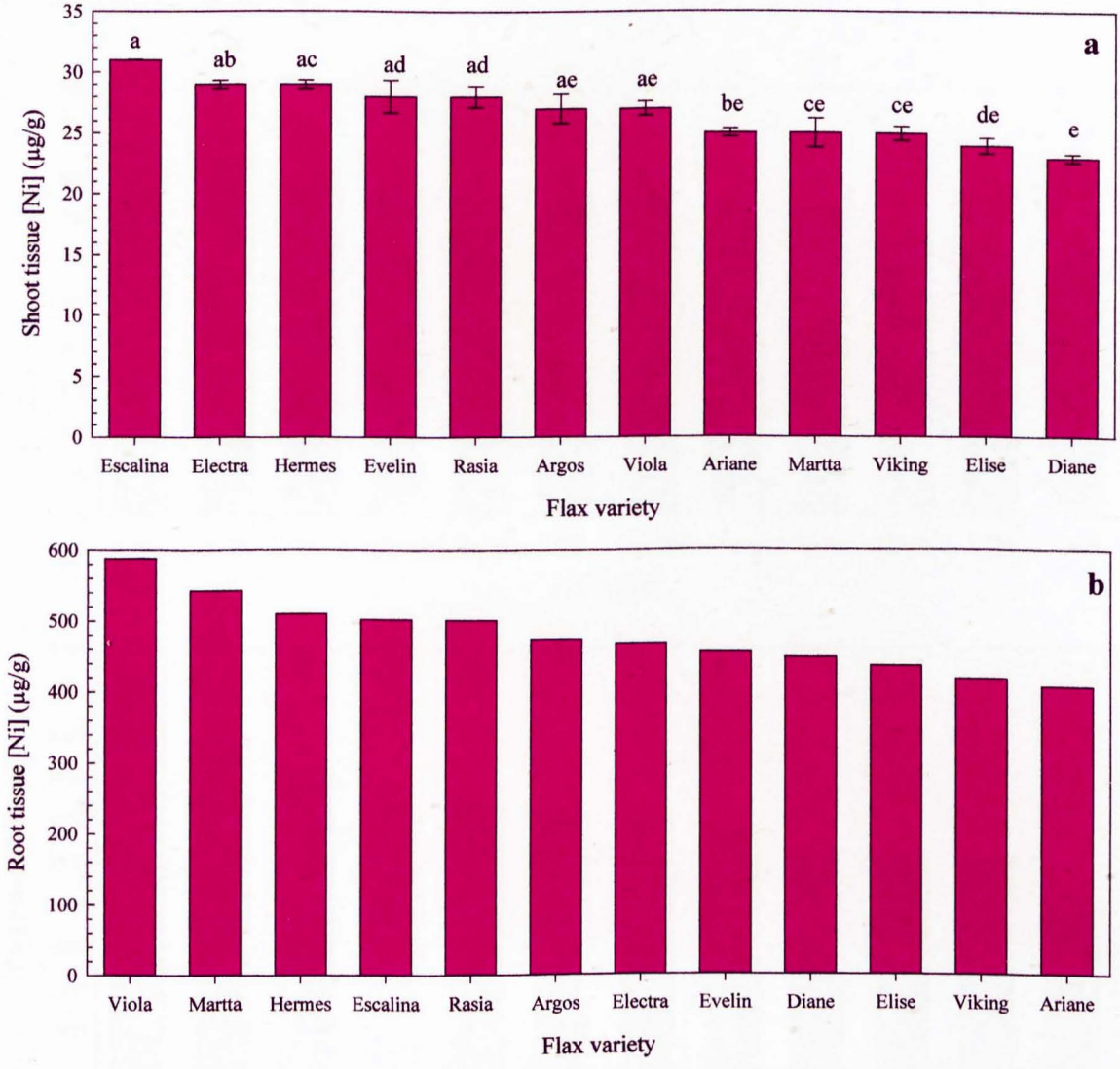

Figure 5.15 Flax tissue Ni concentration. Values shown represent: (a) mean shoot tissue Ni concentration (µg/g)and (b) pooled root tissue Ni concentration (µg/g), for each variety (Section 2.9). Error bars represent SE of the mean. In (a) the values are significantly different, ANOVA. Values sharing the same letter are not significantly different, *t* test with Bonferroni correction, $P>0.05$.

Zinc

Martta, the variety which had the highest shoot tissue Zn concentration, accumulated 440 µg Zn/g, making Zn the metal present in shoot tissue at the highest concentration of all the metals studied (Fig. 5.15a; Table 5.4). The variety with the lowest tissue Zn concentration was Rasia with a shoot concentration 70% that of Martta, giving Zn the second smallest range in shoot tissue concentrations across all the varieties after Ni. In addition to being taken up in shoot tissue at the greatest concentration, of the six metals studied, Zn was also accumulated in the greatest quantity in proportion to the solution concentration, with variety Martta having a shoot concentration 44 times the solution concentration (Table 5.4).

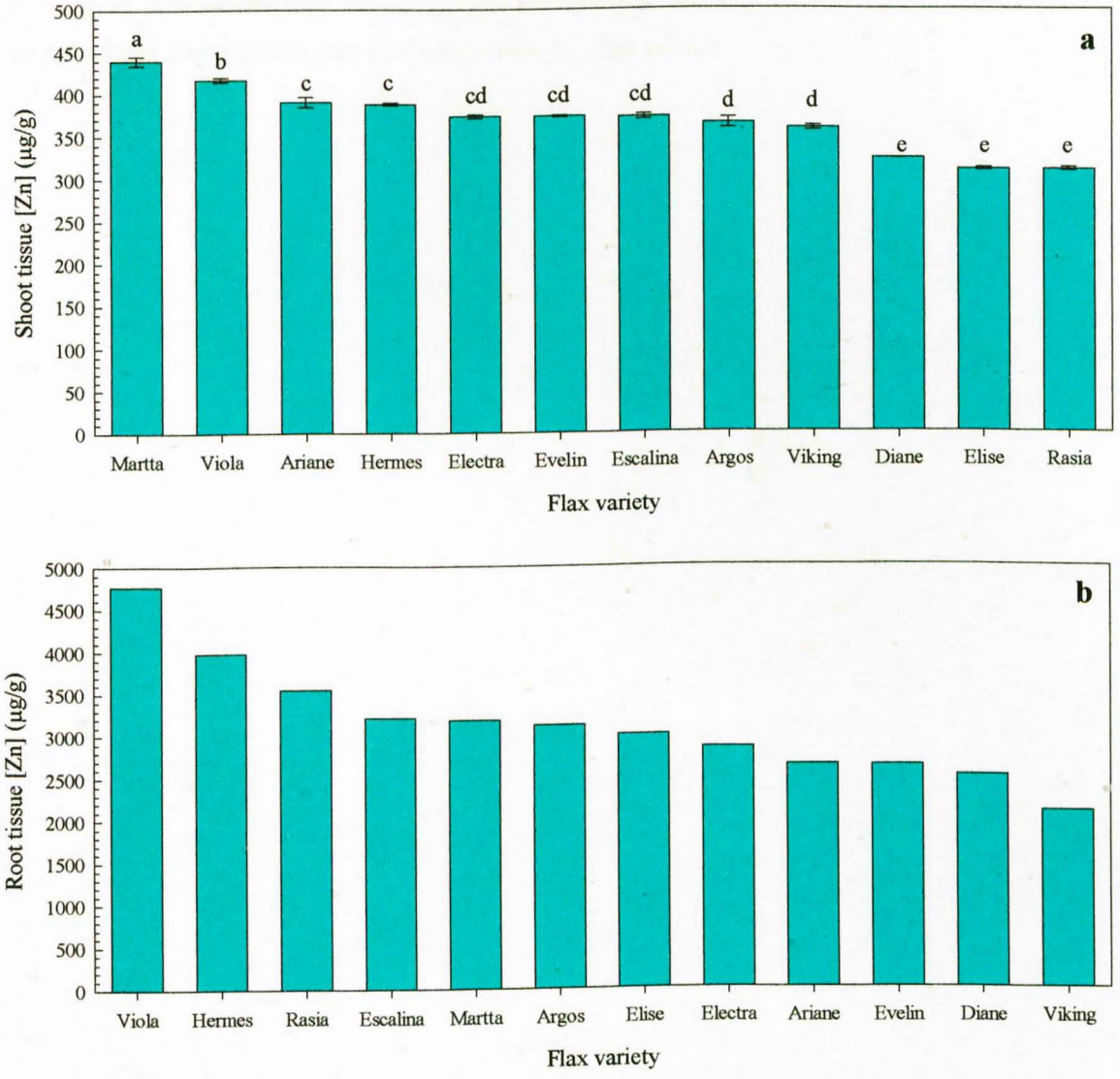

Figure 5.16 Flax tissue Zn concentration. Values shown represent: (a) mean shoot tissue Zn concentration (µg/g) and (b) pooled root tissue Zn concentration (µg/g), for each variety (Section 2.9). Error bars represent SE of the mean.In (a) the values are significantly different, ANOVA. Values sharing the same letter are not significantly different, *t* test with Bonferroni correction, $P>0.05$.

Unlike shoot tissue, the uptake of Zn into root tissue had a large range across all varieties. Notably large differences were observed in root tissue Zn concentrations between each of the top three root Zn accumulating varieties: Viola, Hermes and Rasia, which had root tissue Zn concentrations of 4766 μg/g, 3978μg/g and 3549μg/g respectively. The variety with the lowest root tissue Zn concentration (Viking) only accumulated 44% of the Zn accumulated by Viola.

The mean root Zn uptake of all varieties was 3126 μg/g which was the second highest root tissue metal concentration of all the metals after Pb (Table 5.5). This was an accumulation of 313 times the solution Zn concentration which was greater than the root accumulation of Cr and Pb but lower than the root accumulation of Cu, Cd and Ni. Zinc had the lowest root:shoot uptake ratio, of all the metals, indicating that Zn was the metal most efficiently translocated from root tissue to shoot tissue by flax plants.

5.2.2.1 Metal uptake summary

An accumulation of each of the metals in both root and shoot tissue for all of the flax varieties grown in the experiments was observed with the exception of Cr. Chromium was the only metal not to be detectable in shoot tissue although this metal was accumulated in root tissue by all the varieties. This meant that, at the solution concentrations considered, even the least mobile metals (except Cr in shoot tissue) were concentrated in both below and above ground flax tissues.

For all the metals except Cu, uptake to shoots was significantly influenced by the variety considered, and like growth response, the optimum variety for uptake varied with the metal considered. The variety Viola, however, was in the top three accumulators of metal into shoot tissue for all metals but Ni, and for root tissue for all metals but Cu (Fig. 5.11-5.16).

Table 5.4 Flax varieties with the highest tissue metal concentrations for root and shoot tissues. Data shown are the varieties with the highest tissue metal concentrations for shoots and roots expressed as: the tissue metal concentration (μg/g) and the accumulation of metal (accum.) expressed as the number of times the mean tissue metal concentration is greater than the solution metal concentration.

Element	Shoot concentration			Root concentration		
	Variety	μg/g	accum.	Variety	μg/g	accum.
Cadmium	Viola	191	38×	Viola	3051	610×
Chromium	-	-	-	Hermes	148	148×
Copper	Viola	23	23×	Escalina	1537	768×
Lead	Hermes	243	6×	Elise	9506	238×
Nickel	Escalina	31	31×	Viola	588	588×
Zinc	Martta	440	44×	Viola	4766	477×

For Cu, Ni and Zn, a gradual decline in shoot tissue metal concentration from the variety with the highest to the lowest shoot tissue metal concentration was observed (Fig. 5.12a, 5.14a, 5.15a). For two of the metals, the variety with the highest shoot tissue metal concentration had a shoot uptake > 10% of the next highest uptake: Viola-Cd and Hermes-Pb systems took up > 16% and 50% more metal, respectively, than the other varieties grown in the Cd and Pb solutions (Fig. 5.10a, 5.13a). The tissue concentration of the flax varieties alone, however, did not indicate which metals were most efficiently removed from the matrix. For an indication of how efficiently flax removed each metal, root and shoot tissue metal concentrations had to be considered relative to the matrix metal concentrations (Table 5.5).

Table 5.5 Metal concentrations for root and shoot tissue as a mean over all flax varieties. Data shown are: the mean metal concentrations of shoot and root tissues (μg/g); the accumulation (accum.) expressed as the number of times the mean tissue metal concentration is greater than the solution metal concentration and the ratio of mean root:shoot tissue metal concentrations.

Element	Shoot concentration		Root concentration		Root:shoot ratio
	μg/g	accum.	μg/g	accum.	
Cadmium	151	30×	2496	499×	17
Chromium	-	-	118	118×	-
Copper	17	8×	1196	598×	70
Lead	145	4×	6719	168×	46
Nickel	27	27×	479	479×	18
Zinc	368	37×	3126	313×	8

Solution to shoot mobility in decreasing order: $Zn > Cd \geq Ni \gg Cu > Pb > Cr$

Root to shoot mobility in decreasing order: $Zn > Cd \geq Ni \gg Pb \gg Cu > Cr$

The distribution patterns of metals between flax root and shoot tissues, as a mean across all varieties, was such that distinct groups of metals were observed. The behaviours of Cd, Ni and Zn were similar to each other but distinct from those of Cr, Cu and Pb. Although Cd, Ni and Zn had different shoot tissue concentrations, their mean tissue metal loading was 27–37 times the solution metal loading (Table 5.5). Metal accumulation in root tissue, like shoot tissue, was similar for all three metals with a mean across all varieties of between 300 and 500 times the solution concentration. Cadmium, Ni and Zn had root:shoot tissue concentration ratios of < 20 indicating that these metals were readily transported from the roots to shoots. The root:shoot tissue concentration ratios for Cd and Ni were very similar. Zinc was more efficiently transferred from roots to shoots than all of the other metals, with a ratio half that of Cd and Ni.

The situation for the second group of metals (Cr, Cu and Pb) was more complex (Table 5.5). Like the previous group of metals discussed, the shoot tissue concentrations varied. However, Cu and Pb were accumulated at a much lower proportion of the solution concentration than Cd, Ni and Zn, at a shoot tissue metal accumulation of four–eight times the solution concentration. Chromium was distinct from all the other metals as it was not detected in shoots. Chromium and Pb were both accumulated in similar proportions by flax roots. Mean root tissue concentrations of 118 and 168 times the solution concentration, respectively, made these the lowest root–metal accumulations observed. Copper differed

from the other two metals in this group in that flax was able to accumulate ~ 600 times the solution Cu concentration in its root tissue. This was the greatest root tissue metal accumulation of all the metals in the study. Chromium, Cu and Pb were less efficiently transferred from roots to shoots than Cd, Ni and Zn (Table 5.5). These findings agree with the uptake and compartmentalisation of Cd, Ni and Zn versus Cr, Cu and Pb in trees reviewed by Pulford and Watson (2002).

6 BSO and histidine

The buthionine sulfoximine (BSO) and histidine studies were designed to look at the potential for manipulating flax plant growth and metal uptake responses to metal solutions. Plant responses to a biochemical inhibitor (BSO) or a chelating agent (histidine), after addition to metal solutions, was investigated. BSO is known to inhibit glutathione biosynthesis preventing phytochelatin (PC) formation (Section 1.2.6.2). It is hypothesised that PCs may play a role in removing free Cd from the cytosol. In the absence of PCs, Cd mobility to the plant shoots may be increased by inhibition of root sequestration. Histidine has been implicated in chelating Cu and Ni *in vivo* thereby providing protection against metal toxicity (Kramer *et al.*, 1996; Lee *et al.*, 1978; Wheeler *et al.*, 2001; White *et al.*, 1981). If such additives can be shown to increase the uptake of metals into shoot tissue, these chemical additives could be used to increase the efficiency of metal removal from soils by a phytoremediator crop.

Both the BSO and histidine studies were conducted using a static hydroponic system (Section 2.10, 2.11). The static Kilner jar system was the preferred system used for these studies rather than the NFT system; the volume of solution required for the NFT study would have resulted in excessive cost as BSO is an expensive reagent. Additionally, green house space was limited. The Kilner jar system was advantageous over the NFT system as it used a smaller volume of solution which was subject to less evaporation. Flax variety Viking was used in both the BSO and histidine studies.

6.1 BSO Study

Plant growth and Cd uptake by flax exposed to Cd-containing nutrient solutions with the additional inclusion of BSO, was investigated. This was to establish whether the toxicity of the Cd solution was increased and the mobility of the Cd in the flax plant system was affected by the presence of BSO.

Flax plants were grown in Kilner jars containing the treatments as detailed in Section 2.10. The target Cd concentration was 5 μg/ml and the BSO(a) and BSO(b) target concentrations were 100 μM and 25 μM, respectively. The concentration of 44.5 μM (5 μg/ml) Cd was chosen as this concentration had a sub-lethal toxic effect on flax plants in both the NFT study (Section 5.1) and in the flax varietal response to metal solutions study (Section 5.2). BSO was considered at two concentrations: 0.1 mM (Gussarsson *et al.*, 1996) and 0.025mM. BSO is not a competitive inhibitor of Cd chelation but rather it is an inhibitor of

the chelating agents' biosynthetic pathway (Reese and Wagner, 1987), therefore BSO need not be present at the same molarity as Cd.

6.1.1 Growth response of flax to Cd and BSO

Root and shoot weights were measured to assess the effect of the treatment solutions on biomass production of the flax plants. Root and shoot dry weights and shoot fresh weights were recorded (Fig. 6.1–6.3). For each of these three data sets, the same pattern was observed over the five treatments: the control and BSO only treatments (solutions free of Cd) gave similar yields of plant tissue which were higher than those of the Cd containing solutions (Fig. 6.1–6.3). This similarity of the BSO and control growth responses confirmed that BSO did not effect plant growth in the absence of Cd stress (Gussarsson *et al.*, 1996).

For each of the root and shoot tissue yield measurements, the three Cd-containing solutions had a lower biomass yield than the plants grown in the control solution. The root yields for the plants grown in the Cd-containing solutions were all 0.08 g, equivalent to ~ 77% of the flax root yield in the control solution (Fig. 6.1). The shoot yields of plants grown in Cd-containing solutions were also similar (Fig. 6.2, 6.3). The shoot fresh weights (65–75% of the control yield) had a greater variation between the Cd and BSO + Cd solutions than the dry weight shoot yields (68–75% of the control yield).

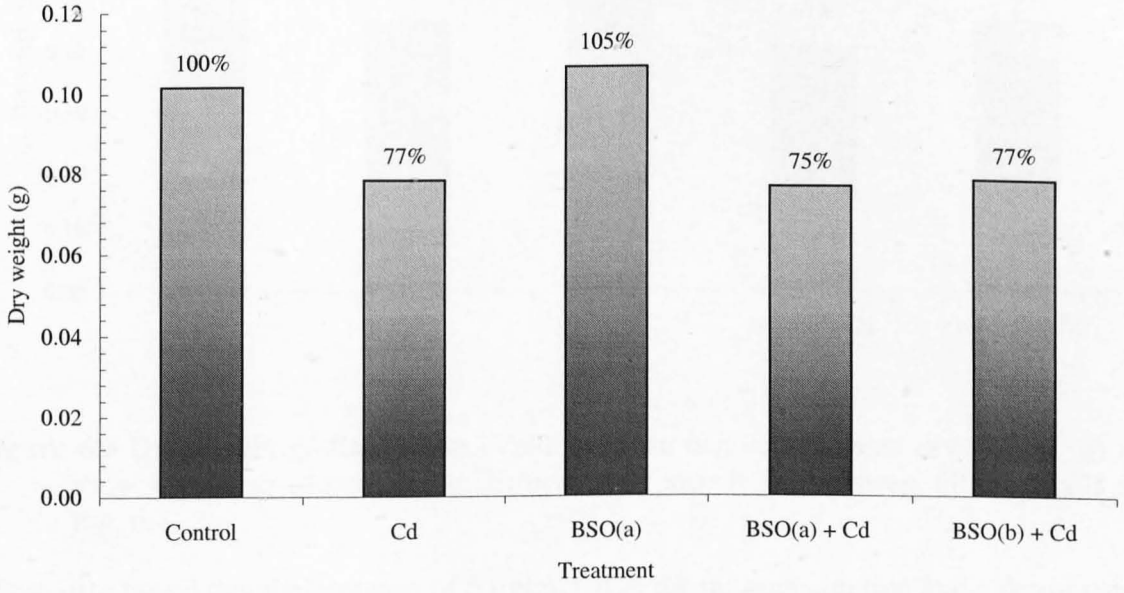

Figure 6.1 Dry weight of flax roots. Values shown are the mean dry weight (g) of flax root tissue per growth collar (pooled root weight from all growth collars/no. of replicate growth collars). Percentages above the bars denote each treatment weight expressed as a percentage of the contol treatment weight.

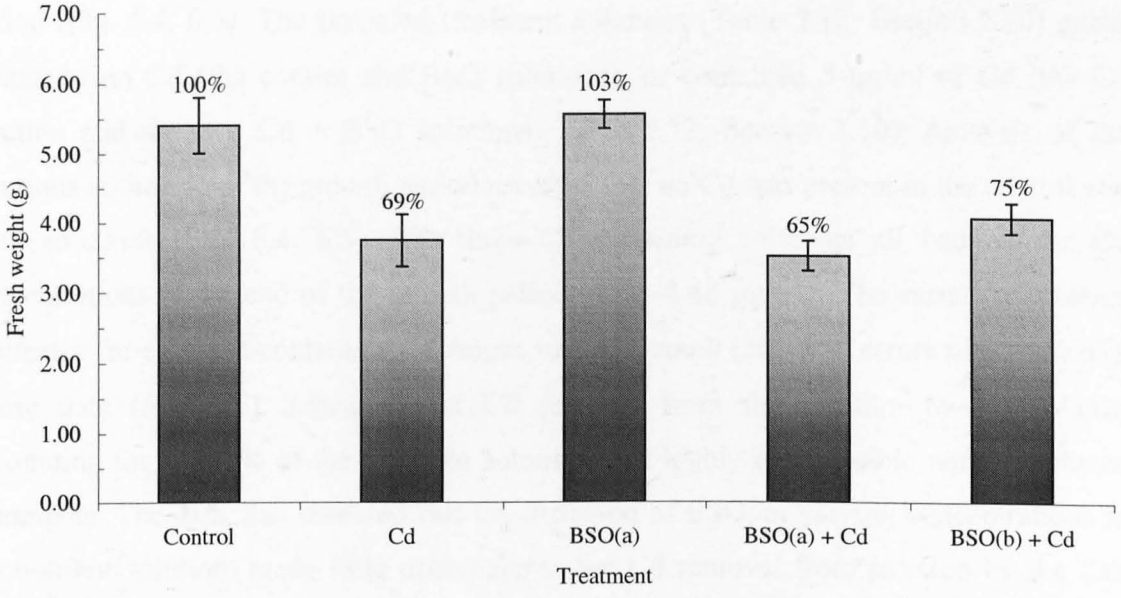

Figure 6.2 Fresh weight of flax shoots. Values shown denote the mean fresh weight (g) of shoot tissue per growth collar. Error bars represent SE of mean. The control and BSO values were not significantly different, nor were the values for Cd-containing solutions (*t* test, $P>0.05$). Other details as Fig. 6.1.

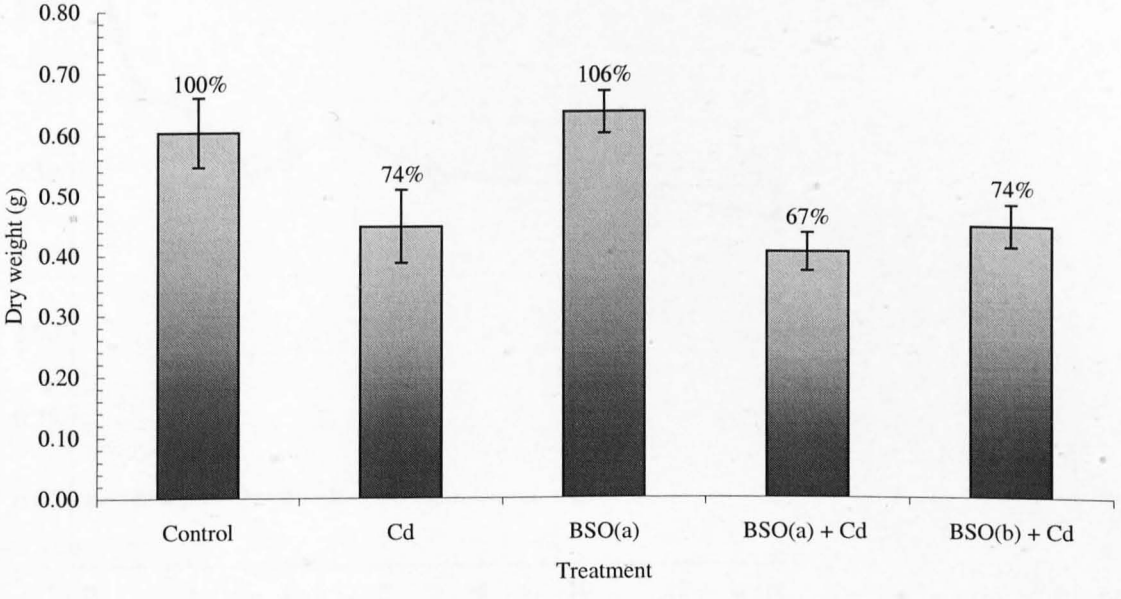

Figure 6.3 Dry weight of flax shoots. Values shown denote the mean dry weight (g) of shoot tissue per growth collar. Error bars represent SE of mean. Other details as Fig. 6.2.

These date reveal that the presence of 5 μg/ml Cd in the nutrient solution had a detrimental effect on the yield of both roots and shoots of flax, reducing the yield by 23–35%. Inclusion of BSO in the nutrient solution had no significant effect on the yield response of the flax plants and did not increase the toxicity of the Cd solution.

6.1.2 Removal of Cd from nutrient solution by flax

The nutrient solution concentration was measured during and at the end of the 14-d growth period (Fig. 6.4, 6.5). The prepared treatment solutions (Table 2.12; Section 2.10) either contained no Cd (the control and BSO solutions), or contained 5 µg/ml of Cd (the Cd solution and the two Cd + BSO solutions, Table 2.12; Section 2.10). Analysis of the solutions at the end of the growth period revealed that no Cd was present in the control and BSO solutions (Fig. 6.4, 6.5). The three Cd-containing solutions all had similar Cd concentrations at the end of the growth period (4.36–4.48 µg/ml). The variation between replicates for each Cd-containing treatment was also small (standard errors of 0.04–0.05). These data (Fig. 6.5) indicated that Cd removal from the solution by flax plants, accounting for 14–15% of the Cd from solution, was highly reproducible within replicate treatments. The data also revealed that the inclusion of BSO, at varying concentrations in the nutrient solution, made little difference to the Cd removal from solution by the flax plants.

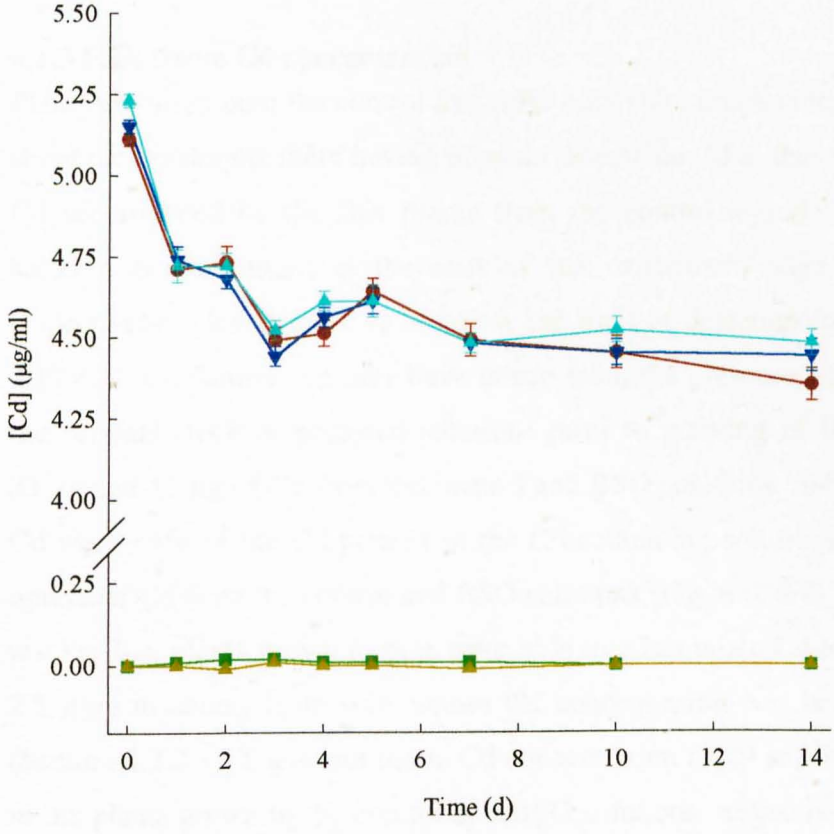

Figure 6.4 Solution Cd concentrations over the 14-d growth period. Data shown denote the mean solution Cd concentrations (µg/ml) in the Kilner jars containing the control solution (—■—), the Cd solution (—●—), the BSO solution (—▲—), the BSOa + Cd solution (—▼—) and the BSOb + Cd solution (—▲—). Error bars represent SE of mean.

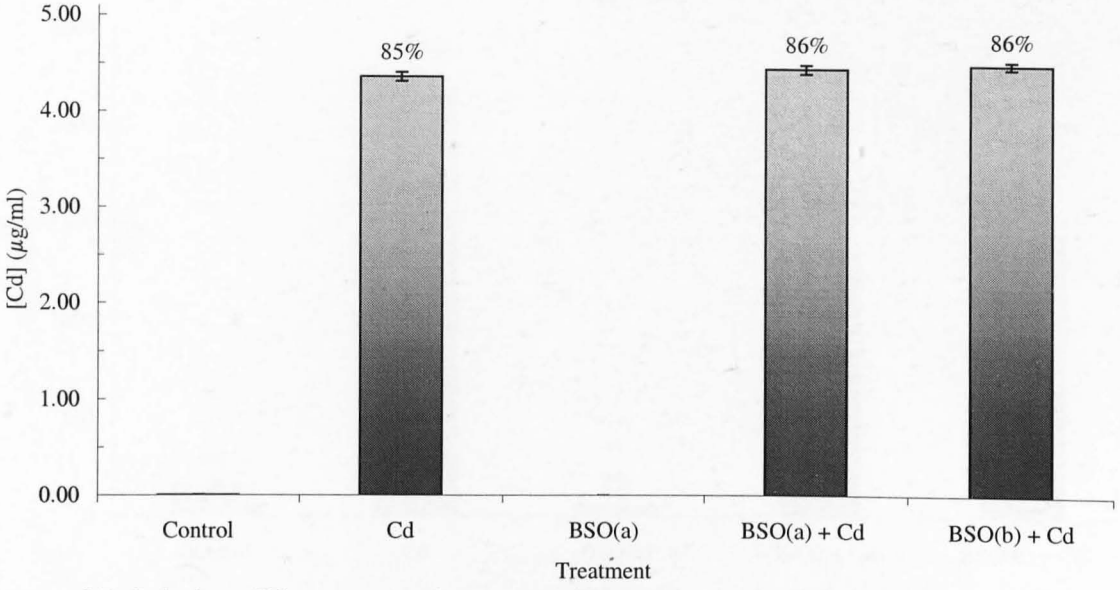

Figure 6.5 Solution Cd concentration remaining at the end of the 14-d growth period. Values shown denote the mean solution Cd concentration (μg/ml) after removal of the plant roots from the replicate Kilner jars. Percentages above the bars represent the final [Cd] expressed as a percentage of the initial [Cd]. Error bars represent SE of mean.

6.1.3 Flax tissue Cd concentration

Plants grown in both the control and BSO only solutions accumulated Cd in their root and shoot tissues despite there having been no detectable Cd in these solutions. The quantity of Cd accumulated by the flax plants from the control and BSO solutions suggested the solution concentrations at the start of the experiment were 0.022 and 0.008 μg/ml, respectively— levels close to or below the limit of detection for Cd (0.02 μg/ml; Section 2.12.4.1). Contamination may have arisen from the presence of small quantities of Cd in the original stock or prepared solutions prior to growing of the plants. Plants removed 33 μg and 13 μg of Cd from the control and BSO solutions, respectively; these weights of Cd were <1% of the Cd present in the Cd-containing solutions (7500 μg). The observed uptake of Cd from the control and BSO solutions (Fig. 6.6, 6.7) was analogous to previous results: flax plants grown in pots were able to accumulate Cd to a tissue concentration of 2.5 μg/g in shoots from soils whose Cd concentration was below the limit of detection (Section 4.2.3.1). The shoot tissue Cd concentration of 29 and 10 μg/g (Fig. 6.7) observed in the plants grown in the control and BSO solutions, respectively, was much higher than that observed in the plants grown in the pot experiment soils (Section 4). The greater tissue Cd concentration observed in the hydroponic system compared to the soil system may have been due a greater availability and mobility of Cd in the Knops solution than the soil.

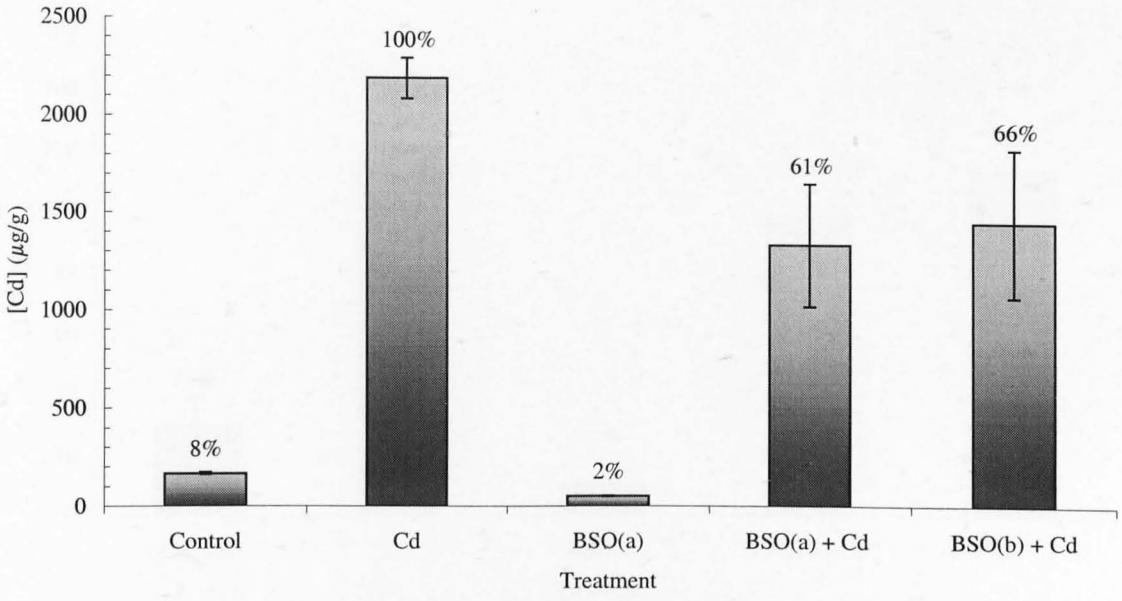

Figure 6.6 Root tissue Cd concentrations at the end of the growth period. Values shown denote the root tissue Cd concentrations (μg/g). Mean values were calculated from analytical replicates drawn from a single root tissue sample pooled from all five replicate growth collars (Section 2.10). Percentages above the bars denote each treatment's Cd uptake expressed as a percentage of the Cd uptake from the Cd only solution. The values for Cd-containing solutions were not significantly different (*t* test, $P>0.05$). Error bars represent SE of mean.

In both roots (Fig. 6.6) and shoots (Fig. 6.7), the flax plants grown in the Cd solution were able to accumulate more Cd than the corresponding BSO + Cd-containing solutions. The flax plants grown in the Cd only solution accumulated 428 times the solution concentration in their root tissue and 24 times the solution concentration in their shoots. The tissue Cd concentrations of 2186 μg/g for roots and 121 μg/g for shoots were slightly lower than the corresponding Cd uptakes observed in the flax screening experiment (Section 5).

The flax tissue uptake data indicated that BSO did not increase Cd uptake either from solution to roots or from roots to shoots (Fig. 6.6, 6.7). In root tissue, Cd uptake in the plants grown in the BSO(a) + Cd and BSO(b) + Cd solutions was reduced by 39% and 34%, respectively, compared to Cd uptake in plants grown in the Cd only solution. The results from each of the Cd + BSO solutions, however, were not significantly different from the Cd only solution. The plants grown the BSO(a) + Cd solution, which had the higher BSO concentration, had a greater reduction in Cd root tissue concentration than the BSO(b) + Cd solution, compared to the Cd only solution.

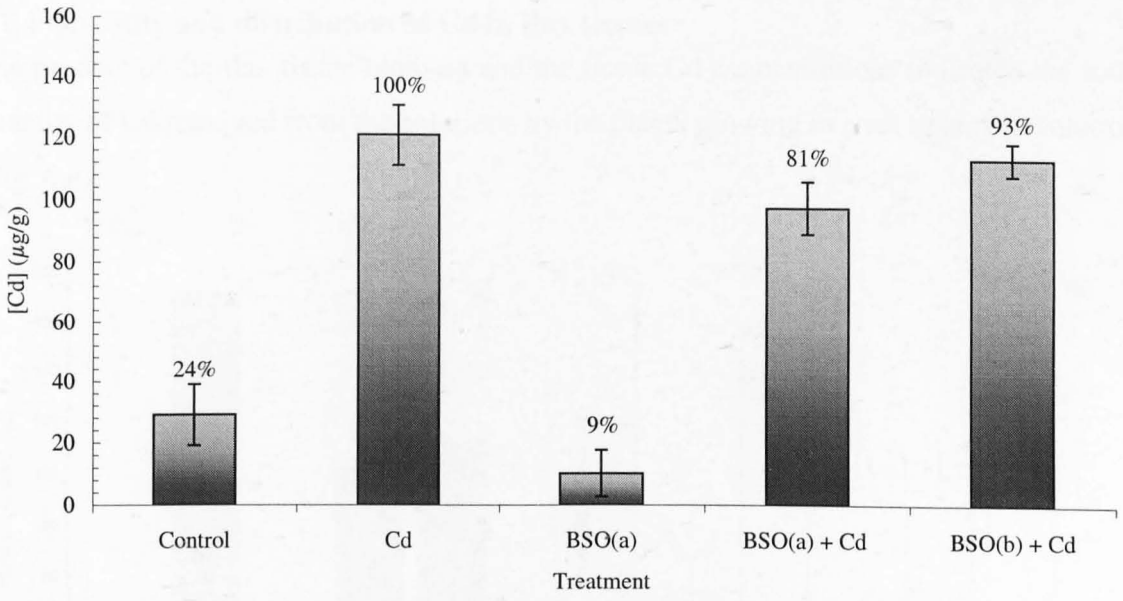

Figure 6.7 Shoot Cd concentration at the end of the growth period. Values shown denote the shoot tissue Cd concentration (μg/g). Percentages above the bars denote each treatment's Cd uptake expressed as a percentage of the Cd uptake from the Cd only solution. The values for Cd-containing solutions were not significantly different (*t* test, $P>0.05$). Error bars represent SE of mean.

A similar situation was observed in Cd uptake in the shoot tissue for the plants grown in the three Cd-containing solutions (Fig. 6.7). There was a reduction in Cd uptake from the Cd solution to the BSO(a) + Cd and BSO(b) + Cd solutions of 20% and 7%, respectively, compared to the Cd uptake of the plants grown in the Cd solution. The results from the Cd + BSO solutions were not significantly different from the Cd only solution. As was indicated by the root uptake data, the reduction in Cd shoot uptake was greater in the plants grown in the more concentrated BSO solution.

6.1.4 Quantity and distribution of Cd in flax tissues

The product of the flax tissue biomass and the tissue Cd concentrations indicated the total quantity of Cd removed from the solutions by the plants growing in each treatment solution (Fig. 6.8).

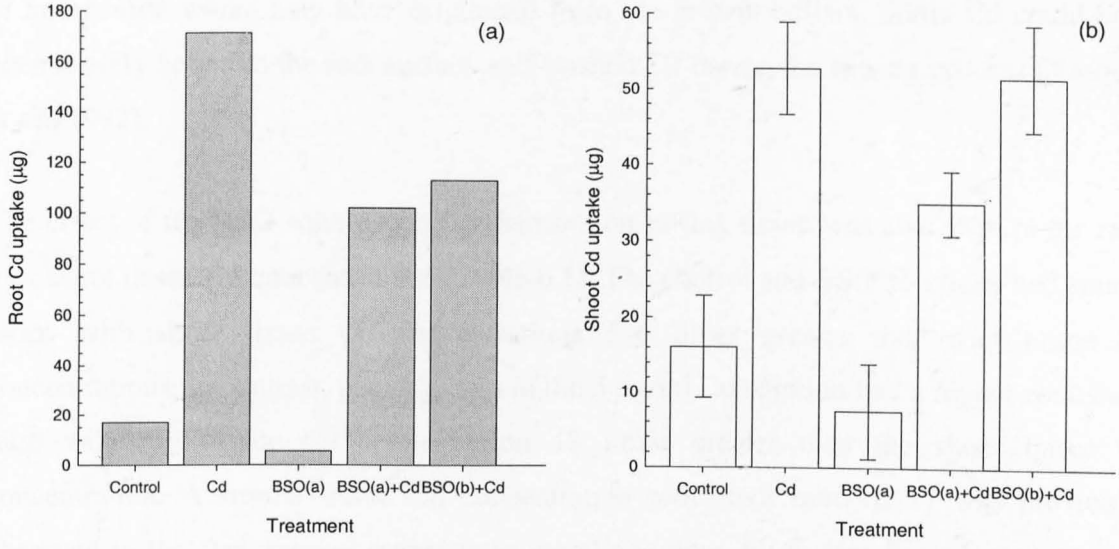

Figure 6.8 Total weight of Cd present in flax tissue. Data represent the total weights of Cd (µg) present in: (a) root tissue (■) and (b) shoot tissue (□). Error bars in (b) represent SE of mean.

The resulting total uptake data mirrored the patterns seen previously with the tissue concentration data (Fig. 6.6, 6.7). Combining plant biomass yield and tissue Cd concentration data resulted in a significant difference in Cd uptake between the Cd only and the Cd + BSO(a) solutions. The quantity of Cd which flax was able to remove from Cd containing solutions and sequester in harvestable plant tissues was reduced in the presence of BSO. The small reduction in shoot tissue concentration together with the modest shoot tissue yield reduction, observed for plants grown in the Cd + BSO(a) solution, combined to have a significant reduction in total Cd uptake compared to the Cd only solution (*t* test $P<0.05$).

The plants growing in the Cd blank solutions, which must have contained trace Cd contamination, were able to accumulate a significant quantity of Cd relative to the solution Cd concentration. Flax was observed to readily take up and translocate Cd at solution Cd concentrations below the limit of detection (Fig. 6.8), but at the higher Cd concentration, the proportion of Cd taken into flax tissues from solution decreased.

In all three Cd-containing solutions, the total quantity of Cd removed from solution could not be accounted for in the plant tissue. In the Cd, BSO(a) + Cd and BSO(b) + Cd

solutions, 1130, 1063 and 1120 μg of Cd was removed from the 1.5 l of solution, respectively, whereas, only 224, 137 and 164 μg was found in the plant tissues, respectively (Fig 6.8). The missing Cd could have precipitated out of solution in the Kilner jars as insoluble salts, Cd–root exudate complexes (Dushenkov *et al.*, 1995) or become adsorbed onto insoluble particles present in the nutrient solution, such as small quantities of vermiculite which may have originated from the growth collars. Some Cd could have been loosely bound to the root surface and washed off during the rinsing process (Vasquez *et al.*, 1992).

The effect of the BSO solution on Cd distribution in flax tissue was also seen in the ratio root:shoot tissue Cd concentrations (Table 6.1). The control and BSO solutions had similar ratios with shoot tissue Cd concentrations 5–6 times greater than root tissue Cd concentrations. In contrast, plants grown in the 5 μg/ml Cd solution had a higher root:shoot ratio with root tissue Cd concentration 18 times greater than the shoot tissue Cd concentration. A similar tissue Cd concentration root:shoot ratio of 17 was previously observed in the flax varietal response to metal solutions study (for flax plants grown in 5μg/ml Cd solution, Table 5.5; Section 5.2.2.1). In the BSO + Cd solutions, the root:shoot ratio was lower than that observed in the Cd solution. Whilst both the root and shoot tissue Cd concentrations were reduced by the presence of BSO, the decrease in the root:shoot ratio was attributable to a greater reduction in root tissue Cd concentration than shoot tissue Cd concentration (Fig. 6.6, 6.7).

Table 6.1 Relative distribution of Cd in flax tissues. Data shown are the ratio root:shoot tissue Cd concentrations of flax plants grown in each treatment solution. Treatment solution concentrations are detailed in Table 2.12; Section 2.10.

Treatment	*Root to shoot ratio*
Control	6
Cd	18
BSO(a)	5
BSO(a) + Cd	14
BSO(b) + Cd	13

Comparison of total biomass production with total Cd uptake revealed the disparity between the distribution of biomass and the distribution of Cd in flax, illustrating some major difficulties in considering flax as a potential phytoremediation crop (Fig. 6.9). Most of the plant biomass, approximately 85%, was in the easily harvestable shoot tissue whereas the majority of the Cd (42-76%) was concentrated in the root tissue. Furthermore, comparison of the root to shoot ratio of the control and BSO solutions with the Cd-

containing solutions indicated that at the higher solution Cd concentration, proportionately less Cd was transported to the shoot tissue. This reduction in translocation efficiency as solution Cd concentration increased would have to be overcome if a successful phytoremediation strategy were to be developed.

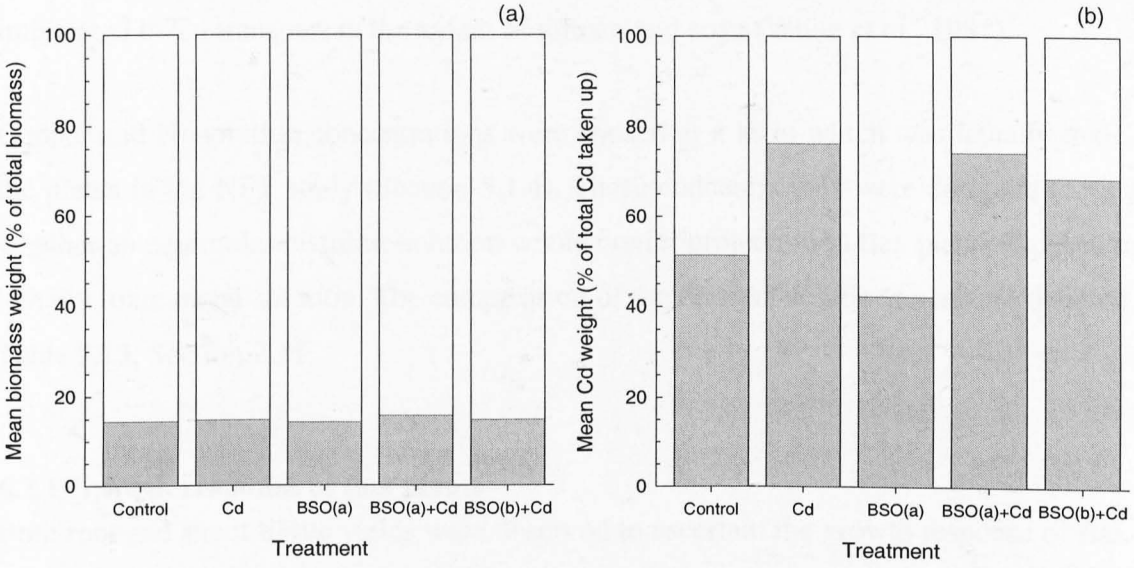

Figure 6.9 Distribution of plant biomass and Cd uptake between flax roots and shoots. Data shown are: (a) the mean weights of root (■) and shoot (□) biomass expressed as a percentage of the mean biomass for each treatment, and (b) the mean weight of Cd present in roots (■) and shoots (□) expressed as a percentage of the total weight of Cd taken up by plants grown in each treatment.

6.1.5 BSO Summary

The inhibition of flax growth by Cd was not increased by the presence of BSO in contrast to previous reports of the response of Cd-tolerant plants to BSO in the presence of Cd (Rauser, 1990). BSO did not significantly affect shoot tissue Cd concentrations at either of the concentrations considered. However, the combined effect of BSO on plant growth and plant tissue Cd concentration, both of which were reduced compared to the Cd only solution, resulted in a significant reduction in the quantity of Cd removed from the solution by sequestration in flax shoots. The flax response to BSO was dependant on BSO concentration as a significant effect was only seen at the higher of the two BSO concentrations used in the study.

The presence of trace quantities of Cd contamination in the control and BSO only solutions allowed comparisons to be made between the mobility of Cd in flax at low and high concentrations. It was observed that proportionally more Cd was present in tissues of flax plants grown in solutions containing Cd below the limit of detection than in tissues of plants grown in 5 μg/ml Cd solution. The root to shoot Cd concentration ratios of the control and BSO only solutions also indicated that Cd is more readily transported to harvestable flax tissues when present at low solution Cd concentrations.

6.2 Histidine study

The purpose of this study was to determine the effect of an addition of the amino acid histidine on the growth and metal uptake responses of flax plants. Kramer *et al.* (1996) reported that supplying non-accumulating species with histidine conferred enhanced tolerance to Ni and resulted in greater transfer of Ni to shoots. Histidine has also been implicated in Cu transport in the xylem of tomato and soya (White *et al.*, 1981).

Copper and Ni solution concentrations were chosen at a level which was lethally toxic to the plants in the NFT study (Section 5.1.4). These concentrations were designed to verify whether an equimolar histidine solution would confer protection to flax plants exposed to a lethally toxic metal solution. The composition of the nutrient solutions used are detailed in Table 2.13, Section 2.11.

6.2.1 Growth response of flax plants

Both root and shoot tissue yields were observed to ascertain the growth response of flax to the treatment solutions (Fig. 6.10–6.12). In each of the treated solutions, the root tissue yield was markedly reduced compared to the yield of flax plants in the control solution (Fig. 6.10). The histidine only solution gave a yield of 59% of the control yield, indicating that histidine, in the absence of heavy metal toxicity, had a deleterious effect on flax root production. The reduction in root yield for the Ni and Cu solutions was greater than that observed in the histidine only solution. The Ni and Cu solutions were not lethally toxic to the flax plants grown in the Kilner jars unlike the flax response to the same concentrations observed in the NFT study (Fig. 5.3; Section 5.1.5). The difference in flax response to the similar concentration in the two different systems may be attributable to the oxidation status of the solution or due to a more sensitive root response under the well aerated rhizosphere conditions of the NFT system. The NFT system differed from the Kilner jar system in that the solution was continually aerated. The roots in the NFT system were also constantly exposed to both nutrient solution and air due to the thin nutrient film in the NFT troughs, unlike the roots in the Kilner jar system which were completely submerged in the nutrient solution and thus exposed to more anaerobic environment.

Flax root yields in the Ni and Ni + histidine solutions were 25% and 41% of the control solution root yields, respectively (Fig. 6.10). These data indicated that histidine attenuated Ni-induced root yield reduction. For the Cu solutions, the situation was reversed. Compared to the control flax root yield, the Cu solution gave a flax root yield of 30% whilst the Cu + histidine solution gave a root yield of 23%, which was the lowest root

yield observed (Fig. 6.10). Thus histidine did not confer any protection to root biomass production in the presence of Cu.

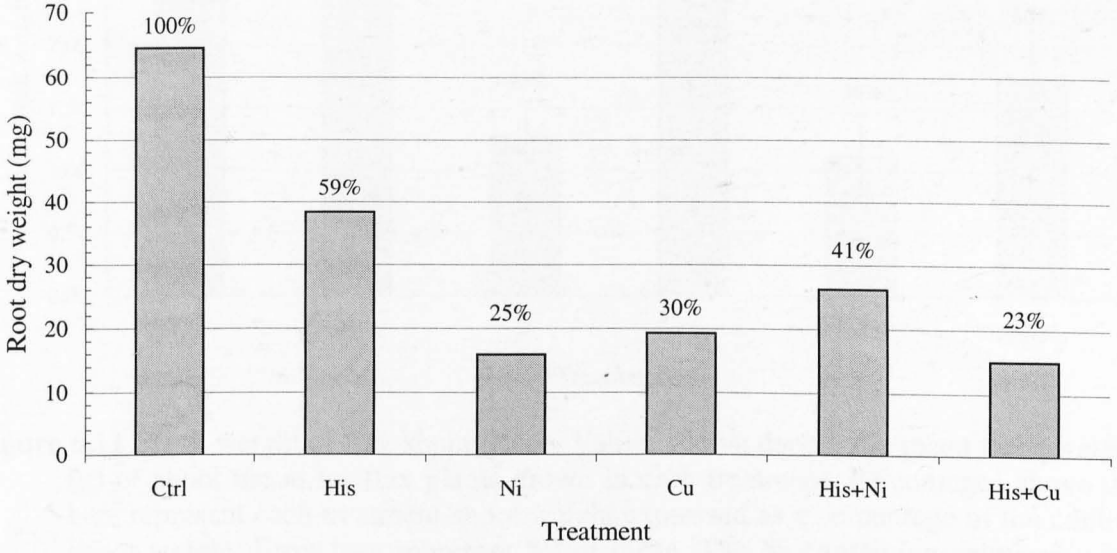

Figure 6.10 Dry weight of flax root tissue. Values shown denote the mean oven dried weight of root tissue (mg) for flax plants grown in each treatment. Percentages above the bars represent each treatment root weight expressed as a percentage of the control root weight. Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

A more complex situation was observed for shoot yield (Fig. 6.11, 6.12). For flax grown in the histidine only solution, there was the same reduction in yield (9%) observed in both fresh weight and dry weight shoot biomass compared to the control plants. In the Cu solution, the reduction in fresh weight shoot yield was also similar to that of dry weight shoot yield compared to the plants grown in the control solution at 19% and 22%, respectively (Fig. 6.11, 6.12). For the remaining solutions (Ni, histidine + Ni and histidine + Cu) the reduction in fresh weight yield, when compared to the control plants, was much greater (~ 2–3 times) than the reduction observed in dry weight yield.

These data indicated that the presence of histidine had a negligible effect on shoot biomass production (Fig. 6.11, 6.12). The reduction in dry weight biomass was unaffected by the presence of histidine in solution as all of the metal-containing solutions reduced the dry biomass production of flax by approximately 20% compared to the control plants.

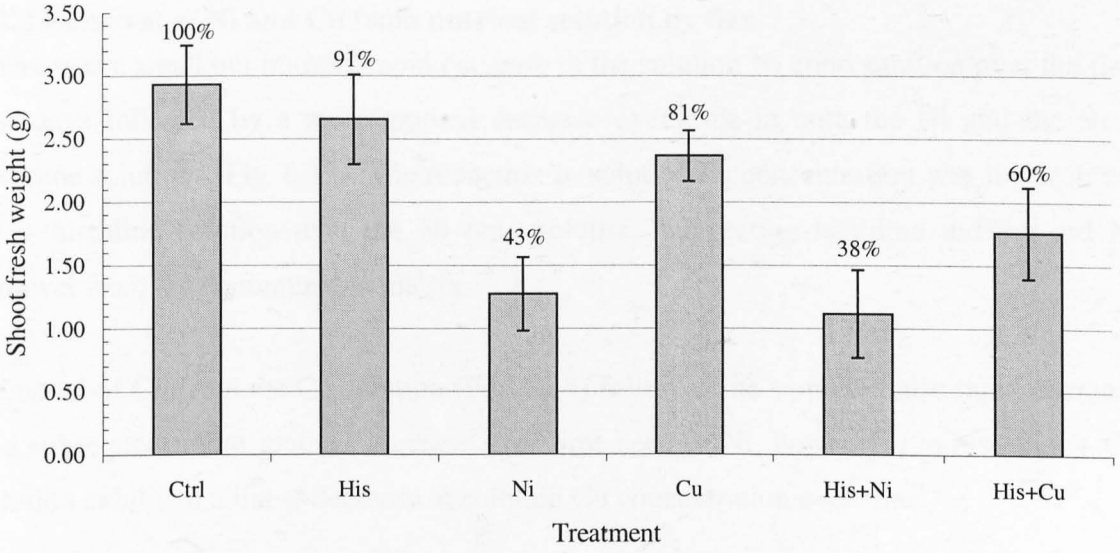

Figure 6.11 Fresh weight of flax shoot tissue. Values shown denote the mean fresh weight (g) of shoot tissue for flax plants grown in each treatment. Percentages above the bars represent each treatment shoot weight expressed as a percentage of the control shoot weight. Error bars represent SE of mean. The Ni-containing solutions were not significantly different nor were the Cu-containing solutions (*t* test, $P>0.05$). Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

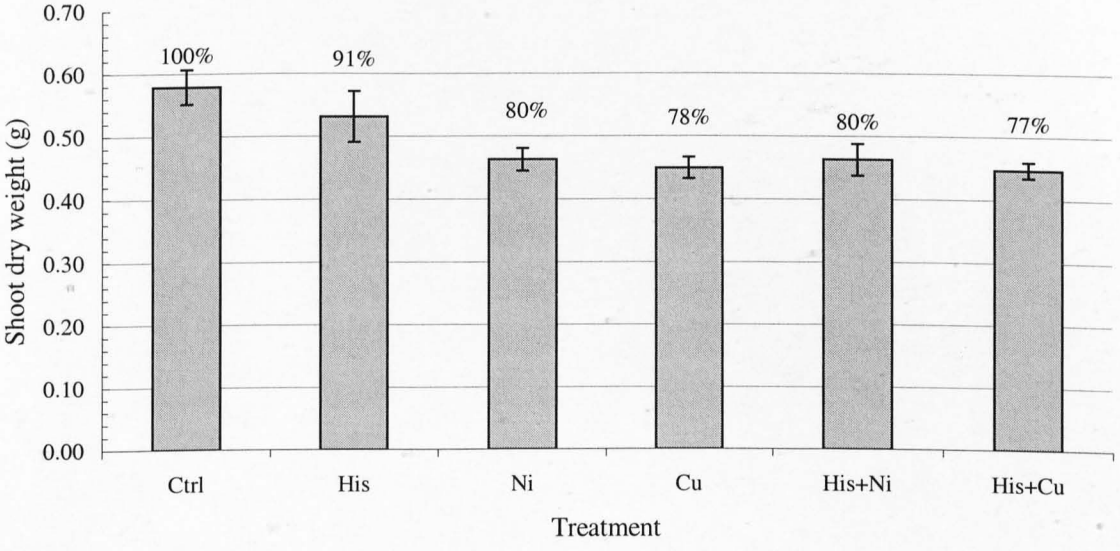

Figure 6.12 Dry weight of flax shoot tissue. Values shown denote the mean oven dried weight (g) of shoot tissue for flax plants grown in each treatment. Other details as Fig. 6.11.

The fresh weight biomass was reduced to a greater extent than the dry weight biomass for both Ni-containing solutions and for the Cu + histidine solution, suggesting Ni had a desiccating effect on the shoot tissue which was not alleviated by the presence of histidine. Furthermore, Cu in combination with histidine also had a desiccating effect on the shoot tissue although neither Cu or histidine had this effect when present in isolation.

6.2.2 Removal of Ni and Cu from nutrient solution by flax

There was a small but initially rapid decrease in the solution Ni concentration over the first few days followed by a more gradual decrease over time in both the Ni and the Ni + histidine solutions (Fig. 6.13). The reduction in solution Ni concentration was lower in the Ni + histidine solution than the Ni only solution, suggesting histidine did not aid Ni removal from the contaminated matrix.

Removal of Cu from the Cu solution (Fig. 6.14) followed the same initially rapid decrease and subsequent more gradual decrease over time seen in Ni, however, the histidine + Cu solution exhibited a linear decrease in solution Cu concentration over time.

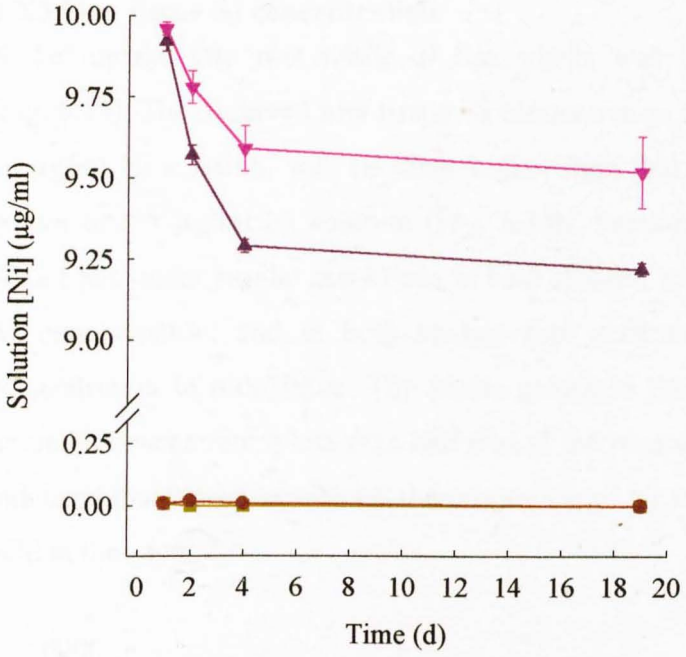

Figure 6.13 Solution Ni concentration during the growth period. Values shown are the solution Ni concentrations (µg/ml) for: the control solution (—■—), the histidine solution (—●—), the Ni solution (—▲—) and the histidine + Ni solution (—▼—). Error bars represent SE of mean. The target concentration for the Ni solution was 9.98 µg/ml (Table 2.13; Section 2.11).

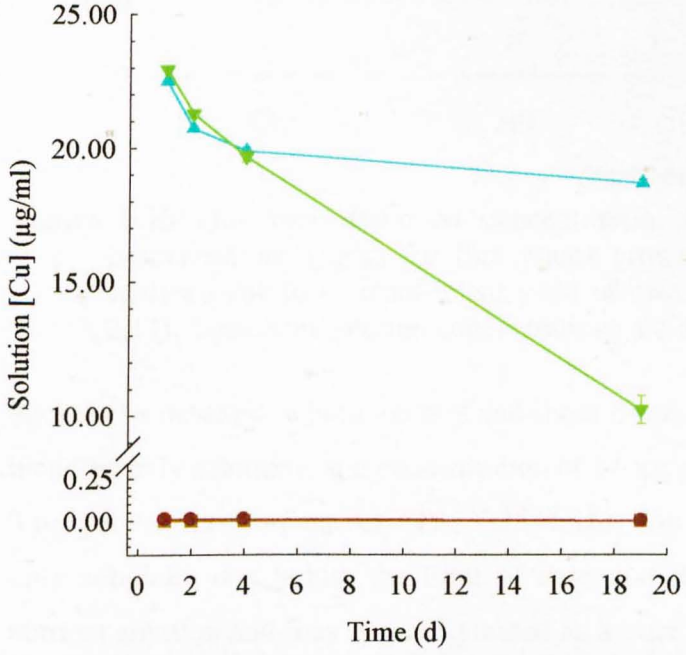

Figure 6.14 Solution Cu concentration during the growth period. Values shown are the solution Cu concentrations (µg/ml) for: the control solution (—■—), the histidine solution (—●—), the Cu solution (—▲—) and the histidine + Cu solution (—▼—). Error bars represent SE of mean. The target concentration for the Cu solution was 21.60 µg/ml (Table 2.13; Section 2.11).

6.2.3 Flax tissue Ni concentration

Nickel uptake into root tissue of flax plants was greatest from the Ni only solution (Fig. 6.15). The observed root tissue Ni concentration of 4992 μg/g, from flax grown in the 10 μg/ml Ni solution, was 10 times higher than that observed previously for flax plants grown in a 1 μg/ml Ni solution (Fig. 5.15b; Section 5.2.2). Flax plants were grown in Kilner jars under similar conditions in both studies, with the exception of the difference in Ni concentration, and in both studies flax accumulated ~500 times the solution Ni concentration in root tissue. The plants grown in the histidine + Ni solution had a root tissue Ni concentration less than half that of the plants grown in the Ni only solution. This indicated that histidine reduced the proportion of Ni from the nutrient solution which was held in the roots.

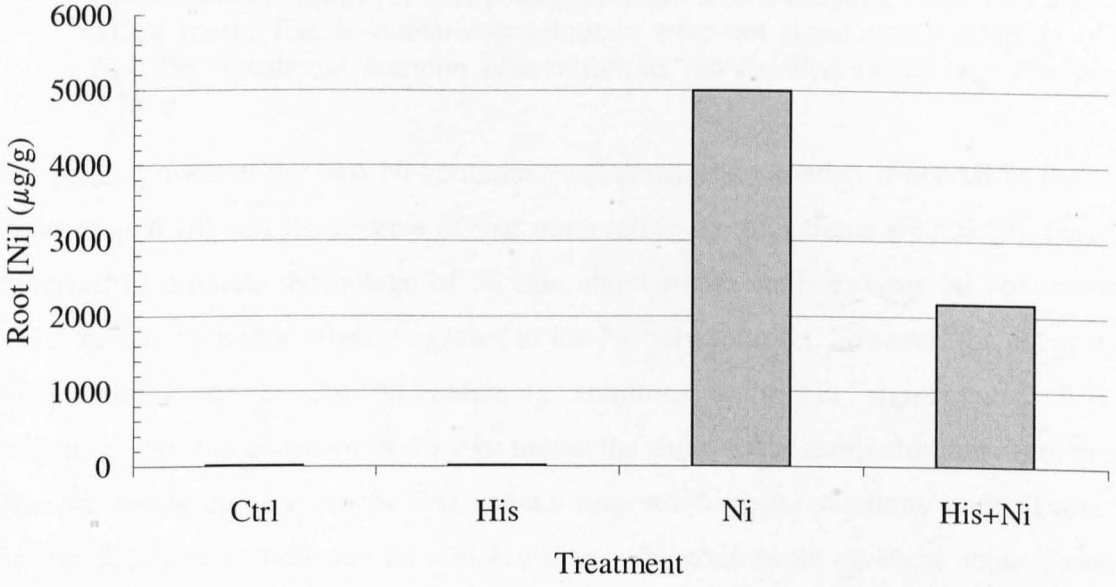

Figure 6.15 Flax root tissue Ni concentration. Data shown are the root tissue Ni concentrations (μg/g) for flax plants grown in each treatment. Data are single analyses due to an insufficient yield of root tissue for replicate analyses (Section 2.11). Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

Nickel was detected in both the root and shoot tissue of the plants grown in the control and histidine only solutions, at a concentration of 14 μg/g and 15 μg/g for roots and 7 μg/g and 3 μg/g for shoots, respectively (Fig. 6.15, 6.16). The Ni present in the control and histidine only solutions was below the limit of detection (0.02 μg/ml; Section 2.12.4.1) in the nutrient solution and may have originated as a trace contamination in the stock solutions. The flax plants grown in the control solution, therefore concentrated the Ni by >700 times in root tissue and by >350 times in shoot tissue.

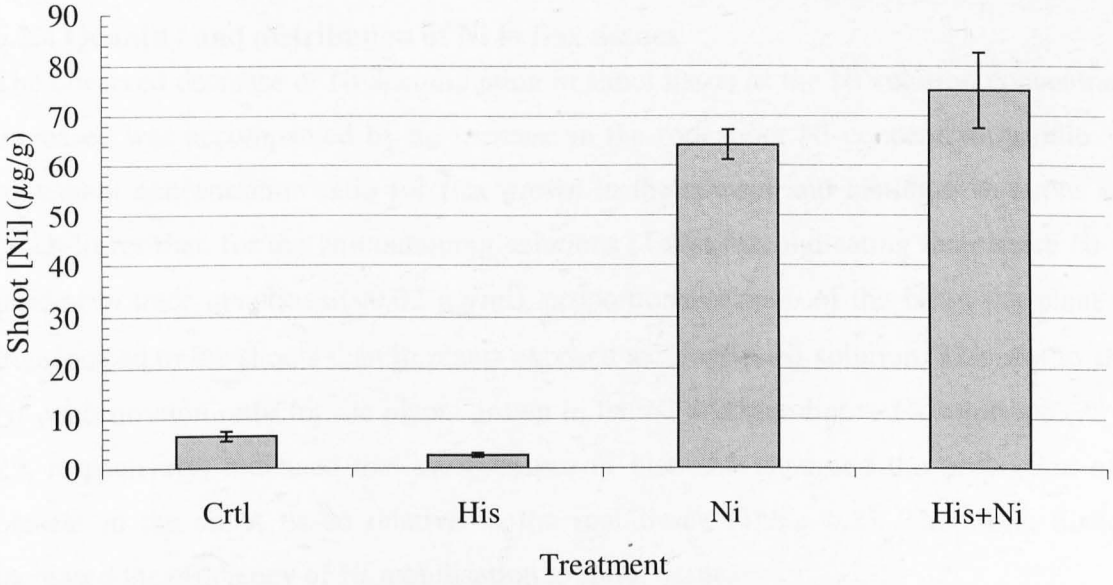

Figure 6.16 Flax shoot tissue Ni concentration. Data shown are the shoot tissue Ni concentrations (μg/g) for flax plants grown in each treatment. Error bars represent SE of mean. The Ni-containing solutions were not significantly different (*t* test, $P>0.05$). Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

For plants grown in the two Ni-containing solutions, the situation observed in the shoot tissue (Fig. 6.16) was the reverse of that observed in the root tissue (Fig. 6.15). Histidine appeared to promote the uptake of Ni into shoot tissue, with a tissue Ni concentration ~17% greater than that of plants grown in the Ni only solution, however, the shoot tissue Ni concentrations in the Ni-containing solutions were not significantly different (Fig. 6.16). As was observed in the root tissue, the shoot tissue concentrations were greater than previously observed in the flax varietal response to metal solutions study (Table 5.5; Section 5.2.2), with both the Ni and histidine + Ni treatments giving a shoot tissue Ni concentration more than double that observed in the earlier study (Fig. 5.15a; Section 5.2.2). The Ni accumulation in the Ni and histidine + Ni plants of 6.5 and 7.5 times the solution Ni concentration, respectively (Fig. 6.16), was lower than the accumulation observed in the flax varietal response to metal solutions experiment of 23–31 times the Ni solution concentration; this was in contrast to the observed root accumulation which was ~ 500 times the solution concentration in both studies. The reduced accumulation of Ni between the flax varietal response to metal solutions study (where the Ni concentration was 1 μg/ml) and the histidine study (where the Ni concentration was 10 μg/ml) indicated that at higher solution concentrations Ni accumulation in shoot tissue was reduced.

6.2.4 Quantity and distribution of Ni in flax tissues

The observed decrease of Ni accumulation in shoot tissue as the Ni solution concentration increased was accompanied by an increase in the root:shoot Ni concentration ratio. The root:shoot concentration ratio for flax grown in the control and histidine solutions were much lower than for the Ni-containing solutions (Table 6.2) indicating that where Ni was present in trace quantities (<0.02 μg/ml), proportionally more of the Ni in the plant was translocated to the shoots than in plants exposed to a higher Ni solution. The root to shoot Ni concentration ratio for the plants grown in the Ni and histidine + Ni solutions (78 and 29, respectively) indicated that the presence of histidine increased the proportion of Ni present in the shoot tissue relative to the root tissue (Table 6.2). Therefore, histidine increased the efficiency of Ni mobilisation to shoot tissue.

Table 6.2 Relative distribution of Ni in flax tissues. Data shown are the ratio root:shoot tissue Ni concentrations of flax plants grown in each solution. Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

Treatment	*Root to shoot ratio*
Control	2
Histidine	6
Nickel	78
Histidine +Nickel	29

The mean root:shoot Ni concentration ratio in the flax varietal response to metal solution study (where the Ni concentration was 1μg/ml) was 18 (Table 5.5; Section 5.2.2.1). This ratio was greater than that observed in the control and histidine solutions but less than that of the Ni and histidine + Ni solutions. Therefore, these data have shown that as the Ni solution concentration increased, translocation of Ni to shoots, as a proportion of total Ni in plant tissue, decreased.

The product of the flax tissue Ni concentration and the flax tissue weight gave the total weight of Ni taken up into the flax tissue (Fig. 6.17). Plants grown in the histidine + Ni solution contained 30% less Ni in their roots than those grown in the Ni only solution. However, the plants grown in the histidine + Ni solution, contained 20% more Ni in their shoots than those grown in the Ni only solution. Of the plants grown in each of the treatment solutions, more than 90% of the plant biomass was present as shoot tissue, with the highest percentage (97%) present in the plants grown in the Ni only solution (Fig. 6.18a). The plants exposed to trace amounts of Ni (the control and histidine treatments) were able to transport 70–80% of the plant tissue Ni to their shoot tissue, however, the plants exposed to the 9.98 mg/l Ni solutions only transported 27–38% of the Ni to their shoot tissues (Fig. 6.18b). Although the histidine + Ni treatment had 16% less

Ni present in the flax tissues than the Ni only solution, the histidine + Ni solution did increase the proportion of Ni present in shoot tissue compared to the Ni only solution. This indicated that histidine reduced the overall plant Ni uptake but increased the proportion of the Ni which reached the shoot tissue.

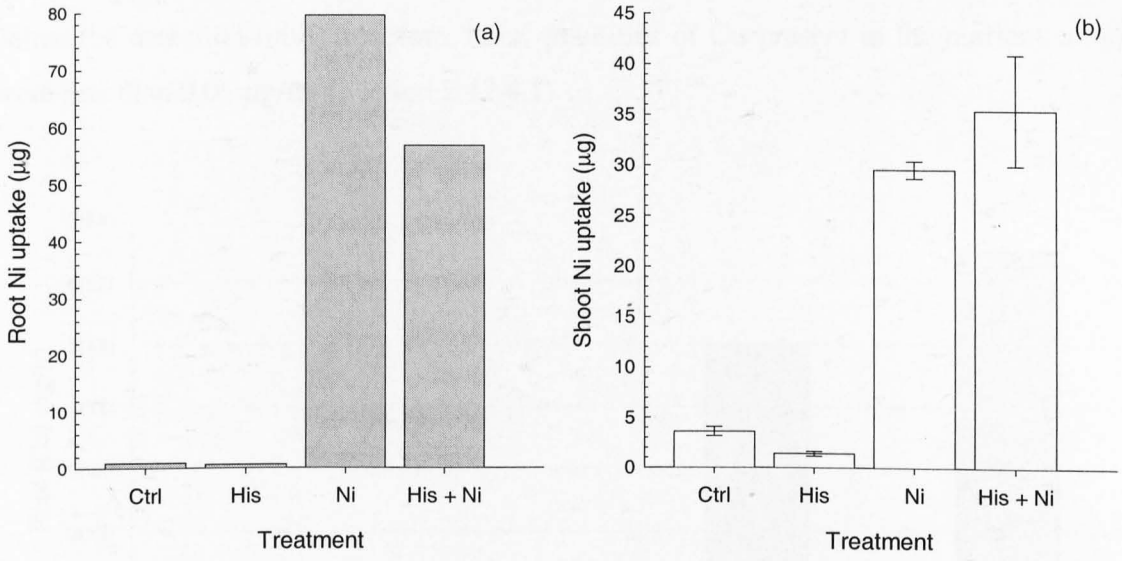

Figure 6.17 Total weight of Ni present in flax tissue. Data represent the total weight of Ni (μg) present in: (a) root tissue (▪) and (b) shoot tissue (□). Error bars in (b) represent SE of mean. Shoot Ni uptake from the Ni-containing solutions was not significantly different (*t* test, $P>0.05$). Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

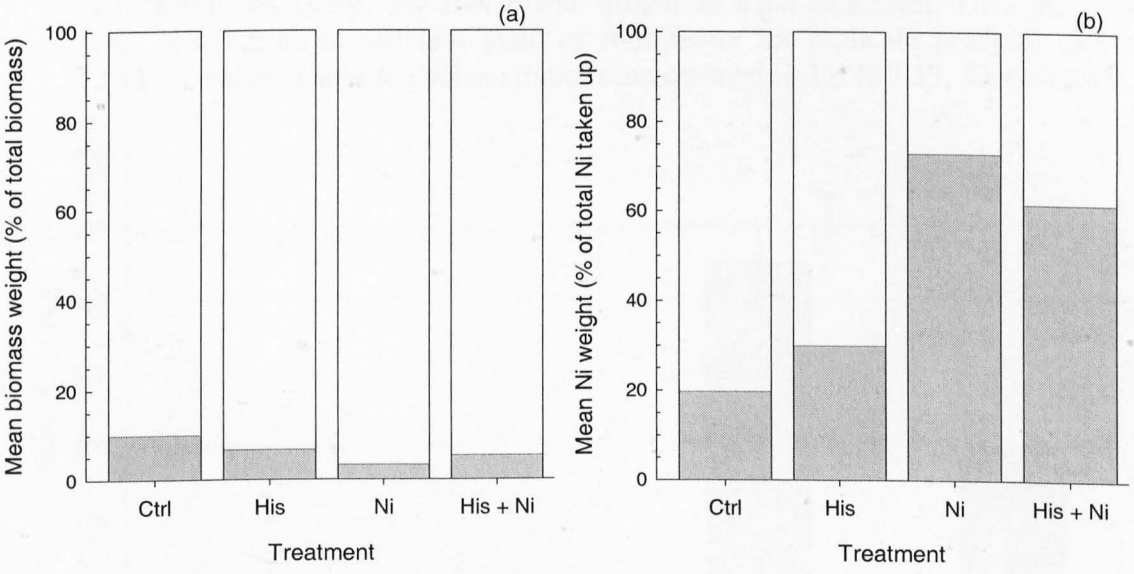

Figure 6.18 Distribution of plant biomass and Ni uptake between flax roots and shoots. Data shown are: (a) the mean weights of root (▪) and shoot (□) biomass expressed as a percentage of the mean flax biomass for each treatment, and (b) the mean weight of Ni present in roots (▪) and shoots (□) expressed as a percentage of the total weight of Ni taken up by plants grown in each treatment. Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

6.2.5 Flax tissue Cu concentration

Copper, like Ni, was detected in root and shoot tissue of the plants grown in the control and histidine only solutions at 86 and 39 μg/g for roots and 14 and 12 μg/g for shoots, respectively (Fig. 6.19, 6.20). The solution Cu concentration for these treatments was below the detection limit, therefore, trace quantities of Cu present in the nutrient solution were less than 0.05 μg/ml (Section 2.12.4.1).

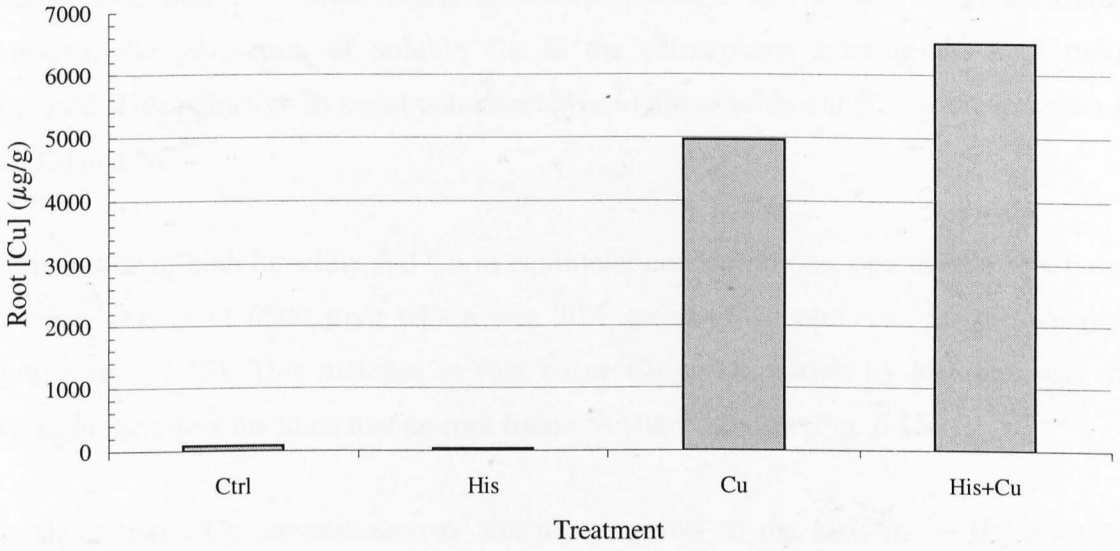

Figure 6.19 Flax root tissue Cu concentration. Data shown are the root tissue Cu concentrations (μg/g) for flax plants grown in each treatment. Data are single analyses due an insufficient yield of root tissue for replicate analyses (Section 2.11). Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

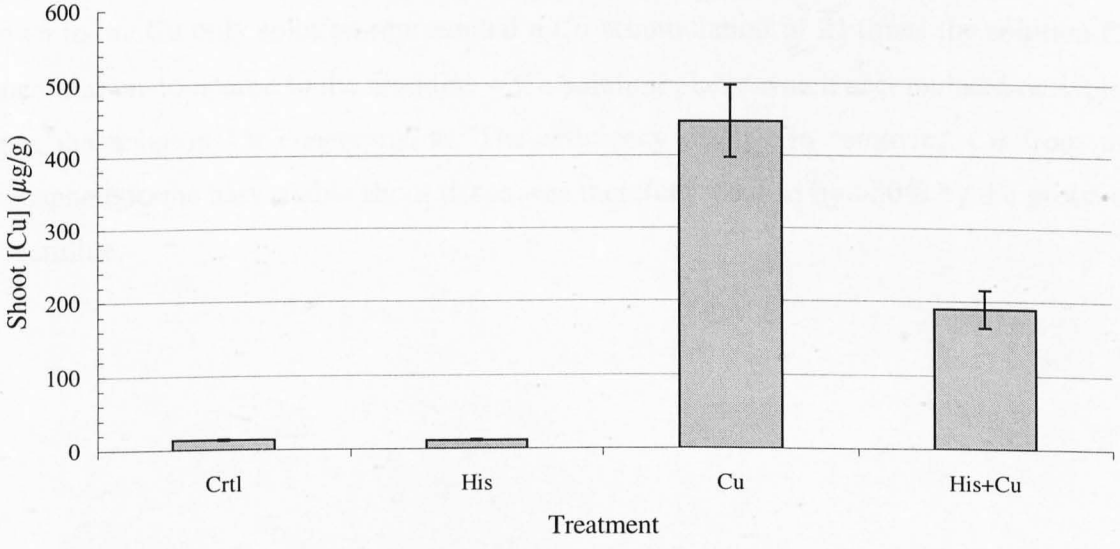

Figure 6.20 Flax shoot tissue Cu concentration. Data shown are the shoot tissue Cu concentrations (μg/g) for flax plants grown in each treatment. Error bars represent SE of mean. The Cu-containing solutions were significantly different (t test, $P<0.05$). Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

Flax plants grown in the Cu only solution were able to accumulate 4964 μg/g Cu in their roots (Fig. 6.19), a concentration similar to the root Ni concentration of the plants grown in the Ni only solution (Fig. 6.15). However, the flax root tissue Cu concentration was an accumulation of 230 times the Cu solution concentration (22 μg/ml), which was less than half the accumulation of Ni by root tissue of flax. The flax root tissue Cu concentration was recorded in previous experiments as 1196 μg/g which was 598 times more concentrated than the 2.0 μg/ml Cu solution (Section 5.2.2). Furthermore the accumulation of Cu in the root tissue of the plants grown in the control solution was >1700 times the solution concentration. These results combined indicated that as the Cu concentration increased, the proportion of soluble Cu in the rhizosphere entering the root tissue decreased. This reduction in metal uptake relative to the solution concentration was seen in both Cd and Ni.

The presence of both histidine and Cu in equimolar concentrations, produced a root tissue Cu concentration of 6500 μg/g which was 30% greater than observed for the Cu only solution (Fig. 6.19). This increase in root tissue Cu concentration by histidine was the reverse of the effect histidine had on root tissue Ni concentration (Fig. 6.15).

The shoot tissue Cu concentration of the plants grown in the histidine + Cu solutions (190 μg/g) was less than half that of the plants grown in the Cu solution (447 μg/g) (Fig. 6.20). Thus, the presence of histidine and Cu together at equimolar concentrations was observed to significantly reduce Cu transport to shoot tissue, the reverse of the effect histidine had on Ni transport to shoots. The shoot tissue Cu concentration of the plants grown in the Cu only solution represented a Cu accumulation of 21 times the solution Cu concentration compared to the histidine + Cu solution plants which accumulated only nine times the solution Cu concentration. The efficiency of flax in removing Cu from the rhizosphere to the harvestable shoot tissue was therefore reduced by >50% by the presence of histidine.

6.2.6 Quantity and distribution of Cu in flax tissues

The observed reduction in efficiency of Cu transport in the presence of histidine resulted in a root:shoot tissue Cu concentration of 34 for the histidine + Cu solution compared to 11 for the plants grown in the Cu only solution (Table 6.3). The root:shoot Cu concentrations in the control and histidine solutions of six and three, respectively, indicated that when Cu was present in trace quantities, flax plants efficiently translocated the metal from the root to the shoots. In the flax varietal response to metal solutions experiment the root:shoot Cu concentrations observed for the plants exposed to 2 μg/ml Cu was 70 (Table 5.5; Section 5.2.2.1) which was greater than the ratio in both the plants exposed to only trace amounts of Cu and the plants grown in the 22 μg/ml Cu solutions. The combination of these results only partially agreed with the pattern observed in both Cd and Ni of decreasing translocation from roots to shoots with increasing metal solution strength. The proportion of plant accumulated Cu present in shoot tissue was greater in the plants exposed to trace quantities (<0.05 μg/ml Cu) than more contaminated metal solutions (22 μg/ml), however, the lowest translocation was observed in previous work at a low Cu concentration (2 μg/ml; Section 5.2.2.1).

Table 6.3 Relative distribution of Cu in flax tissues. Data shown are the ratio mean root:shoot tissue Cu concentrations of flax plants grown in each solution. Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

Treatment	*Root to shoot ratio*
Ctrl.	6
His	3
Cu	11
His+Cu	34

The total quantity of Cu removed by roots of the flax plants grown in the Cu only solution (96 μg) was similar to that of the histidine + Cu solution (98 μg) (Fig. 6.21a). The similarity of the Cu removal by roots in the two Cu-containing solutions was owed to an increase in root tissue Cu concentration in the plants grown in the histidine + Cu solution (Fig. 6.19) being counterbalanced by a lower root yield in these plants compared to the plants grown in the Cu only solution (Fig. 6.10). Thus, histidine increased root tissue Cu concentration but inhibited root biomass production resulting in no net effect on Cu removal from solution by roots.

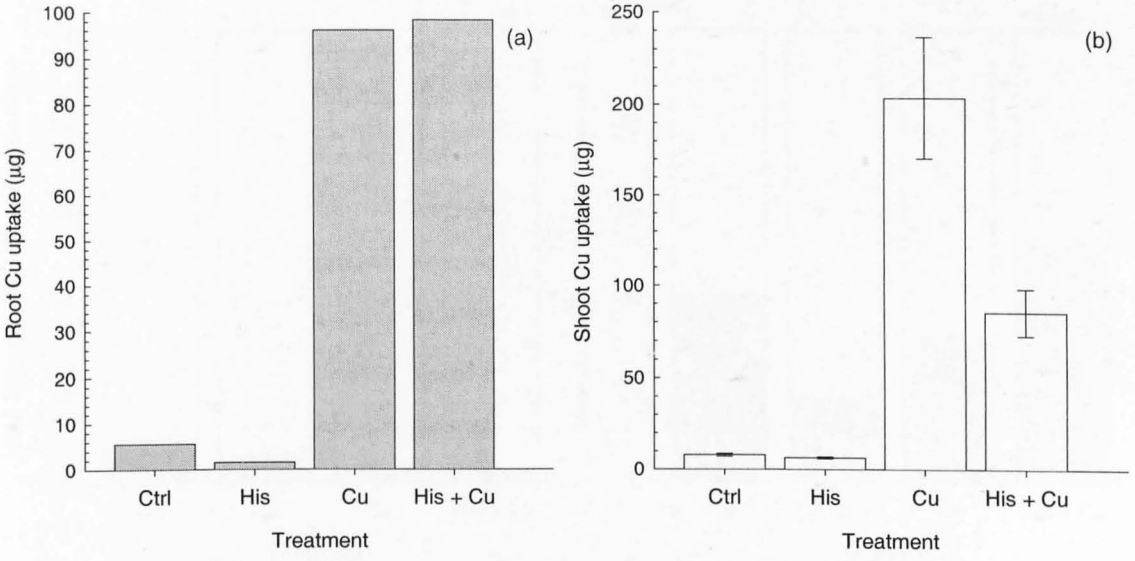

Figure 6.21 Total weight of Cu present in flax tissue. Data represent the total weight of Cu (μg) present in root tissue (a ■) and shoot tissue (b □). Error bars represent SE of mean. Shoot uptake of Cu from the Cu-containing solutions were significantly different (*t* test, $P<0.05$). Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

Due to the similarity in shoot yields for the Cu and histidine + Cu solutions (Fig. 6.12), the total uptake of Cu from these solutions followed the same pattern as the shoot tissue concentrations (Fig. 6.20). The total uptake of Cu by flax shoots in the Cu and histidine + Cu solutions was 203 μg and 85 μg, respectively (Fig. 6.21b). Histidine therefore inhibited the quantity of Cu flax plants were able to remove from solution and translocate to their shoot tissue. The effect of histidine on Cu uptake to shoots was the opposite of the effect on Ni shoot uptake (Fig. 6.17).

Of the plant biomass grown in each of the treatment solutions, ≥90% was shoot tissue (Fig. 6.22a). However, in the histidine, Cu and histidine + Cu solutions the proportion of plant biomass present as root tissue decreased compared to the control. The proportion of the total weight of Cu taken up by flax, which was present in the shoot tissue of the plants exposed to the Cu and histidine + Cu solutions, was greater than the proportion of the total weight of Cd and Ni present in the shoot tissues in the plants exposed to the corresponding Cd and Ni solutions (Fig. 6.9b, 6.18b, 6.22b). The 46% Cu loading present in the shoot tissue of plants grown in the histidine + Cu solution compared to 68% in the plants exposed to the Cu only solution (Fig. 6.22b) illustrated the reduction in phytoremediation efficiency of the flax–Cu system caused by histidine. This was the only solution where the plant shoot tissue Cu loading was less than half of the total plant Cu uptake.

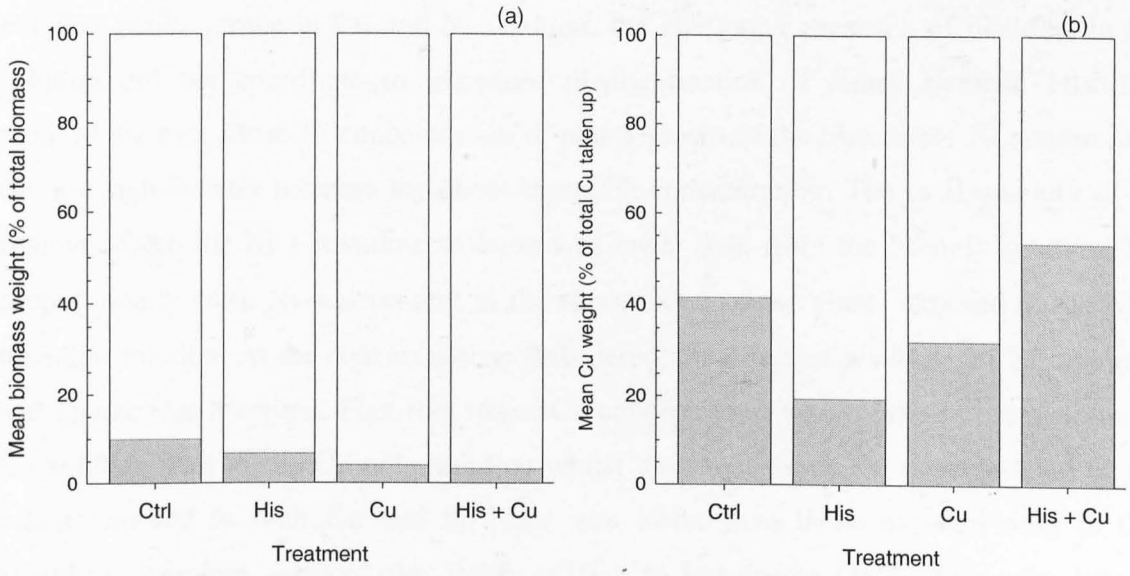

Figure 6.22 Distribution of plant biomass and Cu uptake between flax roots and shoots. Data shown are: (a) the mean total weights of root (▩) and shoot (□) biomass expressed as a percentage of the flax mean biomass for each treatment, and (b) the mean weight of Cu present in roots (▩) and shoot (□) expressed as a percentage of the total weight of Cu taken up by flax plants grown in each treatment. Treatment solution concentrations are detailed in Table 2.13, Section 2.11.

6.2.7 Histidine summary

As was seen with Cd in flax tissues (Section 6.1.4), the total quantity of metal, both Ni and Cu, removed from solution (Fig. 6.17, 6.21) was not accounted for in flax tissues: 86–99% of the metal removed from solution was not accounted for in flax tissues. Like Cd, the missing Cu and Ni may have precipitated out of solution, become adsorbed onto insoluble particles or lost during the sample washing process. The difference in removal of Cu from solution (Fig. 6.14) between the Cu only and the Cu + histidine solutions was not reflected in Cu uptake by the plants grown in these solutions (Fig. 6.21). Together, these data suggest that histidine increased Cu removal from solution but did not increase Cu uptake into flax tissue.

The growth response of flax plants in the combined Ni and histidine solutions (Fig. 6.10–6.12) suggested that, with the exception of some protection against Ni-induced root yield reduction, histidine did not play a role in protecting flax tissues from the yield inhibiting effects of Ni. These results contradicted those of Krämer *et al.* (1997), who reported that histidine, when supplied in solution, conferred Ni tolerance to a non-tolerant plant species (*Alyssum montanum*). The growth response of flax plants in the Cu solution was also not affected by the presence of histidine. Several of the treatments reduced the moisture content of the flax plants (Fig. 6.11, 6.12; Ni, His + Ni, His + Cu), however, during the 19-d growth period of the experiment, this desiccation did not impair dry biomass production.

For flax plants grown in Cu and Ni solution, the equimolar presence of histidine in the solution did not contribute to increased phytoextraction of either element. Histidine reduced the root tissue Ni concentration of plants grown in the histidine + Ni solution but did not significantly increase the shoot tissue Ni concentration. The total quantity of Ni removed from the Ni + histidine solution was lower than from the Ni only solution, but proportionally more Ni was present in the shoot tissue of the plants exposed to the Ni + histidine solution. At the concentrations considered, the effect of histidine on Ni tolerance and uptake was marginal. Flax root tissue Cu concentration was greater in the histidine + Cu solution than in the Cu only solution whilst the shoot tissue Cu concentration of the plants exposed to both Cu and histidine was lower than those exposed only to Cu. Histidine, therefore, reduced the ability of flax to translocate Cu to its' above ground biomass possibly via Cu–histidine complex sequestered in the root tissue.

The presence of trace quantities of Ni and Cu contamination in the control and histidine only solutions allowed comparisons to be made between the mobility of the elements in flax at low and high concentrations. It was observed that proportionally more Ni and Cu were present in tissues of flax plants grown in solutions containing each element below the limit of detection than in tissues of plants grown in more concentrated metal solutions. The root to shoot metal concentration ratios of the control and histidine only solutions also indicated that both Ni and Cu were translocated with greater efficiency to harvestable flax tissues when present at low solution metal concentrations. These findings were in line with the behaviour observed for Cd at low and high solution concentrations (Section 6.1.6).

7 Discussion

Industrial activities have resulted in the contamination of land, particularly in and around populated areas (Scottish Executive, 2001). Contaminated land is a source of pollutants which could adversely affect both human health and the wellbeing of the environment, in addition to preventing economic regeneration of such land and adjoining areas (SEPA, 2001). The remediation of the many contaminated sites both in Scotland and globally is an important goal for current and future generations (Scottish Enterprise, 2001). Phytoremediation has the potential to be a sustainable and economic solution to the problem of contaminated land (Salt *et al.*, 1998).

Phytoremediation, firstly provides a plant cover, which prevents migration of dust and particulate matter off-site (Stomp *et al.*, 1993; Vangronsveld *et al.*, 1995) and secondly, can remove or detoxify soil contaminants (Cunningham *et al.*, 1996; Pulford *et al.*, 2001, Lasat, 2002). Phytoremediation of metal contaminated land requires the plants to take up and subsequently compartmentalise the contaminant metals in harvestable tissues (Kumar *et al.*, 1995). To date, hyperaccumulators (Krämer *et al.*, 1997; L'Huillier and Edighoffer, 1996; Reeves *et al.*, 1996) and high biomass crops, including some brassica species and trees, have been investigated as phytoremediator crops for metals (Blaylock *et al.*, 1997; Pulford and Watson, 2002; Robinson *et al.*, 1999), but little interest has been paid to other industrial crops which may have economic benefit as raw materials for either bio-fuel, industrial oil or fibre industries. In the current work, an assessment was made of four potential phytoremediator crop species: flax, miscanthus, nettle and oilseed rape. In order to evaluate the suitability of each plant species as a phytoremediator, their response to matrices containing metals commonly associated with contaminated land: Cd, Cr, Cu, Pb, Ni and Zn (Alloway, 1995; Neale *et al.*, 1997; Nriagu and Pacyna, 1988; Ross, 1994) was observed. This work considers plant species, which, like short rotation coppice, have the ability to generate an income but, unlike trees and in common with other herbaceous species, including hyperaccumulators, have the ability to generate plant biomass for harvest annually.

For a phytoremediation strategy to be successful, the plant species of interest must be able to establish itself in the contaminated matrix a phase which has been highlighted as the period when plants are most sensitive to metal toxicity (Pulford and Watson, 2002; Punshon and Dickinson, 1999).

Germination was investigated to elucidate if plant behaviour at this initial growth stage was an indicator of plant tolerance to metal contamination. The germination response of flax and oilseed rape seeds, in specially created micro-growth chambers, revealed that germination was unaffected by Cd, Cu, Pb or Ni at concentrations of 500 μg/ml and by Zn at 1000 μg/ml, but was reduced in Cr at 1000 μg/ml by 5–25%. At higher concentrations, flax seeds were able to initiate germination but did not germinate successfully in Cd, Cu and Ni. Additionally, seeds which successfully germinated exhibited poor growth with the exception of those exposed to Pb.

The oilseed rape and flax seeds' ability to germinate in all but the highest solution concentrations suggested that that these seeds were able to tolerate highly contaminated solution concentrations. However, this conclusion was not supported by subsequent results: the stunted shoot lengths of germinated flax plants, the poor emergence and survival data of flax and oilseed rape plants grown in the T1 and T2 soils (with the exception of the Pb soil), and the lethally toxic solution concentrations found in the NFT study. Germination data alone did not provide a guide to the plants' ability to survive exposure to contaminated soils. Using flax as a model system, a more accurate predictor of growth response of plants to metal polluted soils may be the shoot lengths of seedlings germinated in metal containing solutions. This proposal is supported by the case of Pb, where the combined germination and shoot length results for Pb in the germination study were in agreement with the emergence and survival rate of Viking in the Pb_{T1} soil.

Establishment of the rhizomatous plants was observed as part of the pot experiments. The emergence and survival data observed for the miscanthus and nettle rhizome pieces, as well as for the flax seeds, in the T1 and T2 soils indicated that plant responses varied both between metals and between concentrations of the same metal.

Miscanthus was the species most tolerant of the highly contaminated T1 and T2 soils of Pot Experiment One in terms of establishment and survival. All but two miscanthus treatments (Cd_{T2} and Zn_{T2}) had plants surviving until the end of the growth period, furthermore, in several of the miscanthus–soil systems, all the plants that initially emerged survived to the end of the growth period. The remaining miscanthus–, nettle– and flax–soil systems in Pot Experiment One exhibited various toxicity symptoms. Toxicity ranged from: emergence of plants with only partial mortality prior to the end of the growth period; emergence of plants all of which subsequently died prior to the end of the growth period, as was the case for all species grown in the Cd_{T4} soil; failure of plants to emerge as in the two flax–Ni systems. Unlike flax and nettle, none of the treatments were sufficiently toxic

to prevent miscanthus shoot emergence as all treatments initially produced some miscanthus shoots. Following subsequent growth of the emerging seedlings, sewage sludge and Pb_{T1} soils had the highest yields, relative to the control plants, for all three species. The lack of toxicity found in the Pb soils may have been due to the low mobility of Pb in the soil system (Epstein *et al.*, 1999; Lasat 2002). In the Ni_{T1} and Ni_{T2} soils, however, no nettle or flax plants survived, and miscanthus growth was also poor. In the T3 and T4 soils of Pot Experiment Two all of the species were able to establish and survive to the end of the growth period indicating that these more marginally contaminated soil metal concentrations were not prohibitively toxic for plant growth. For flax, the concentrations found to be lethally toxic in the NFT study were in agreement with the poor survival of flax plants in the T1 and T2 soils. The T1 and T2 soils had $CaCl_2$-extractable soil concentrations greater than the concentrations found to be lethal in all but two soils, Cu_{T1} and Pb_{T1}, which were also the soils which had the best flax survival.

Potential phytoremediator species should not only have the ability to become established in the contaminated matrix but subsequently produce sufficient plant biomass to act as a sink for sequestered metals. The highest tissue metals concentrations recorded in the plant species grown in the highly contaminated soils were 868–969 μg Zn/g in the miscanthus–Zn_{T1} soil system and 852–919 μg Cd/g in the nettle–Cd_{T1} soil system. These uptakes were much higher than for any of the other species–soil systems. The Cd and Zn uptakes from these soils compared favourably with uptake of these metals by tree species grown previously in the same soils (McGregor, 1999). In particular, nettles had a tissue concentration between 2–10 times greater than poplar, pine and willow grown in the Cd_{T1} soil. In the case of Zn, miscanthus was able to accumulate approximately the same tissue Zn concentration as the poplar species (which was more than the pine species but less than the willow species) considered by McGregor (1999). In addition to the high metal concentration in the above ground tissue, the yield of the Zn_{T1}–miscanthus plants was also high, making the Zn_{T1}–miscanthus system the most promising potential candidate for phytoremediation observed in the Pot Experiment One soils.

Total tissue metal concentrations in the marginally contaminated soils were greatest in the nettle–Zn_{T3} and –Zn_{T4} soils. In these plant–soil systems the tissue Zn concentrations of 1385–1937 μg/g exceeded the maximum plant tissue Zn uptake recorded in the more contaminated T1 and T2 soils. The nettle tissue Zn concentrations observed in the T3 and T4 soils and the miscanthus tissue Zn concentrations in the T1 soil, were higher than those reported for non-accumulating willow clones but were lower than those reported for the hyperaccumulator *Thlaspi caerulescens* (Baker *et al.* 1994; Brown *et al.*, 1995a; Pulford *et*

al., 2002; Punshon and Dickinson, 1997a & b; Riddell-Black, 1994) Oilseed rape tissue Cu concentrations of the plants grown in the Cu_{T3} and Cu_{T4} soils (50 μg/g and 38 μg/g, respectively) also exceeded the aerial tissue concentrations of all species surviving in the Cu_{T1} soil, and was greater than the Cu concentrations reported for willow clones. For Cd (at 52 μg/g), Cr (at 18 μg/g) and Cu, the maximum tissue metal concentration was recorded in the oilseed rape plants. The species with the greatest tissue Pb and Ni in the Pot Experiment Two soils was nettle at 107 μg/g and 190 μg/g, respectively, which represented relatively high shoot tissue concentrations of these metals (Pulford *et al.*, 2002; Punshon and Dickinson, 1997a).

The behaviour of individual metals in highly contaminated soils differed between plant species; for instance, in the Cd_{T1}–nettle system, the Cd was very mobile, present at > 250% of the soil total metal concentration, and there was slightly more Cd in the leaves than stems. In contrast, Cd mobility in the Cd_{T1}–miscanthus system was much lower, with Cd present at < 60% of the soil total metal concentration and the leaf tissue Cd content > 10 fold that of the stem tissue. Behaviour between metals within an individual plant species also differed; for miscanthus grown in the T1 soils, Cd and Pb were deposited preferentially in the leaf tissue whereas Cu, Zn and Ni were deposited equally between stem and leaf tissues, and miscanthus tissue Zn concentrations were five fold greater than the next most accumulated metal. These observations indicated that it is not possible to make generalisations about the quantity of metal taken up or distributed in plants, rather each metal–plant species system must be considered individually.

For miscanthus grown in the T1 soils, the leaf tissue concentrations of Cd and Pb were 11 times and 2.3 times greater than the stem tissue concentration, respectively, suggesting that the plants were actively transporting metal to the leaves rather than passively depositing metal on exchange sites along the transpiration stream. This implies that the metals were transported as soluble metal–chelate complexes such as the Cd–PC complex described for Cd (Grill *et al.*, 1985; Rauser, 1990). The leaf tissue concentration of both Cd and Pb may be attributable to sequestration in particular compartments such as has been observed in trichomes (Martell, 1974; Salt *et al.*, 1995).

Sequential extraction of the highly contaminated T1 and T2 soils revealed that the mobilities of soil metals, relative to the soil total metal concentration, varied according to the strength of the extracting agent. In the most mobile fraction ($CaCl_2$-extractable) the metals fell into two distinct groups where Cd, Zn and Ni were more mobile than Cu, Pb and Cr. In marginally contaminated soils (T3 and T4 soils), the mobilities of the metals followed the same general pattern, for all four plant species considered, as observed in highly contaminated soils. This pattern of metal behaviour has also been observed in trees (Pulford and Watson, 2002; Watson, 2002). In all four plant species, Cr was the least mobile of the metals with tissue Cr concentrations < 5% of the soil total Cr concentration, therefore, at around ICRCL threshold trigger values, Cr was the metal least suited for phytoremediation using flax, miscanthus, nettle or oilseed rape. Chromium is known to have a low soil mobility (Neale *et al.*, 1997) which was confirmed by the minimal quantity extracted by EDTA relative to the total soil Cr content.

Cadmium, Ni and Zn were all accumulated in the harvestable tissue of one or more of the plant species considered. These metals, particularly Ni and Zn, are those most often reported to be hyperaccumulated by plants endemic to metaliferous soils (Gabbrielli *et al.* 1990; L'Huillier and Edighoffer, 1996; Reeves *et al.*, 1996). The greatest accumulation of metal from the marginally contaminated soils observed was the uptake of Cd by flax and oilseed rape. Both plant species accumulated more than ten times the soil total Cd metal concentration (>1000%). Zinc was accumulated by nettle plants at a tissue Zn concentration ~550% that of the soil Zn concentration representing the second highest metal uptake observed. For each of the metals studied, with the exception of Pb, the plant species with the lowest uptake was miscanthus whilst the plant species in which metals were most mobile were oilseed rape and nettle. Oilseed rape and nettle, however, were the two species which consistently had the lowest yield in each treatment and consequently these plant species did not remove the greatest weight of metal from the marginally contaminated soils. Miscanthus was the plant species able to remove the greatest weight of metal from each of the Cr, Cu, Pb, Ni and Zn soils by virtue of it's high biomass relative to the other crops.

The mobility of the metals in the sewage sludge soil–plant systems was much lower than the metal mobilities in the corresponding artificial soil–plant systems. The differences in soil processes between the sewage sludge– and artificial soil–plant systems, responsible for the reduction in metal mobility, had the least effect on Cu. The positive growth response of all plant species in the sewage sludge soil, despite its high metal content, demonstrated that soil metal loading alone does not determine plant growth response to the soil. The low

metal uptake from the sewage sludge soil, which corresponded to low soil metal concentrations in the $CaCl_2$ extractions, highlighted the important limitation soil metal availability has on the potential for successful phytoremediation (Lasat, 2002).

Plant yield responses to the impoverished soil matrices, used in the marginally contaminated soils compared to the sewage sludge soils, indicated that soil properties such as soil structure and nutrient status may be as important for successful phytoremediation as soil total metal concentrations. Robinson *et al.* (1999) were able to triple the yield of the hyperaccumulator *Alyssum bertolonii* using fertiliser additions. The potential of oilseed rape and nettle to act as phytoremediation crops may be significantly improved by providing the plants with a more favourable growth medium, in particular by using fertilisers, as indicated by the growth response of these species when grown in the sewage sludge soils. The plant tissue metal concentrations achieved by these plant species may warrant further investigation of their potential as phytoremediators.

An initial NFT system was set up, which, with subsequent refinement, produced a system that minimised algal growth and solution evaporation whilst maximising operational efficiency. Investigation of solution metal concentrations required to cause fatality of flax plants in the NFT system found lethally toxic doses of Cr (2 μg/ml), Ni (10 μg/ml) and Zn (100μg/ml) whilst near lethal doses of Cd and Cu were found to be 20 μg/ml and 15 μg/ml, respectively. The $CaCl_2$-extractable metal concentrations in the Pot Experiment One soils were higher than these fatally toxic concentrations, with the exception of the Pb and Cu_{T1} soils. The lethally toxic solution concentrations were considerably lower than the solutions which allowed flax and oilseed rape germination. The values found to be toxic in the NFT study, for Cu and Ni, were similar to toxic levels in barley and ryegrass reported by Davis and Beckett (1978). The lethal concentrations found in the NFT study were used in choosing the metal concentrations for consideration in subsequent hydroponic experiments.

Cieslinski *et al.*, (1996) and Lukipudis, (1994) reported that the efficiency with which flax removed Cu, Pb and Cd from soils was dependent on the variety of flax used. Studies using other species have also shown differences in both metal uptake and yield response to soils with high metal concentrations within species according to the variety/clone used (Huang and Cunningham, 1996; Pulford *et al.*, 2002; Punshon and Dickinson, 1997b; Riddle-Black, 1994). Twelve flax varieties were grown in solutions containing each of the six metals at concentrations below those found to be lethally toxic in the NFT study, these concentrations were chosen to allow identification of varieties both more and less tolerant of the metals than variety Viking used in the NFT study. There was little or no genotypic

advantage in the growth response between flax varieties to the six metals at the concentrations studied. In general the differences in metal uptake between varieties were small, however, there were significant differences in metal uptake into shoot tissue between the flax varieties. Viola and Hermes were the varieties most able to accumulate metal (Cd and Pb, respectively) in their shoot tissues. Viola was the variety most consistently able to accumulate high tissue metal concentrations.

The distribution of metals in the tissues of flax plants grown in hydroponic solutions fell into two groups: Cd, Ni and Zn were transferred readily from roots to shoots and Cr, Cu and Pb were held in roots with a much lower proportion, if any, of the plant metal burden being found in the shoot tissue. Cadmium, Ni and Zn elements were accumulated in shoot tissue at 27–37 times the solution concentration whereas Cr, Cu and Pb shoot concentrations were < 9 times the solution concentration. These findings were in line with the results obtained in the pot experiments.

Plants have been reported to detoxify metals in their tissues using phytochelatins (Section 1.2.6.1). Synthesis of glutathione, an essential precursor of PCs, is known to be inhibited by buthionine sulphoximine (BSO). The simultaneous exposure of plants to both Cd and BSO has been shown to cause Cd-induced toxic stress due to the absence of PCs but plants do not exhibit this stress when exposed to Cd in the absence of BSO (Rauser, 1990; Gussarsson *et al.*, 1996). In contrast, the present work did not show increased Cd-induced growth inhibition in the presence of BSO. The presence of BSO did not significantly affect the shoot tissue Cd concentration of flax plants grown in the BSO + Cd solutions, however, the total quantity of Cd accumulated by shoots was reduced in the plants grown in the more concentrated BSO solution (100 μM). The reduction in total weight of Cd mobilised to shoots suggested that glutathione may play a role in allowing flax plants to take up and translocate Cd from solution to shoot tissue by allowing the formation of a PC–Cd complex.

The data also revealed that the response to BSO was dependent on BSO concentration, as the 100 μM BSO significantly decreased metal uptake to shoots whereas the 25 μM BSO solution did not (Clemens *et al.*, 1999). BSO inhibited the quantity of Cd that can be transported from the rhizosphere to flax shoot tissue. The inhibition of PC production by BSO did not result in greater Cd mobility in the flax plant system. This suggested that PC-mediated Cd transport in flax plants is not localised within single cells or discrete tissues but that PCs may allow transport of Cd from root to shoot tissue where sequestration into vacuoles or specialist tissues may occur (De Knecht *et al.*, 1994; Ow, 1996; Rauser, 1990; Salt *et al.*, 1995; Vogeli-lang and Wagner, 1990). The results reported here agree with the finding of Gussarsson *et al.* (1996) that birch trees exposed to Cd and BSO had reduced Cd accumulation in both root and shoot tissues.

The uptake and transport of Ni by the hyperaccumulator *Alyssum lesbiacum* has been shown to be facilitated by high concentrations of histidine in xylem sap (Krämer *et al.*, 1996). Krämer *et al.* (1996) were also able to confer nickel tolerance to a related non-tolerant plant species, *Alyssum montanum*, by supplying the plant with histidine. Supplying histidine to the non-tolesrant species in the presence of Ni, doubled biomass production and increased xylem transport of Ni. Wheeler *et al.* (2001) also reported that histidine gave *Alnus* protection against Ni toxicity, however, an increase in translocation of Ni from roots to shoots was not observed.

Histidine has also been implicated in Cu tolerance and transport in plants (Lee *et al.*, 1978; White *et al.*, 1981). In this study, the reduction of flax tissue biomass production induced by both Ni and Cu toxicity was unaffected by the presence of histidine. The exposure of flax plants to equimolar concentrations of Ni and histidine did not significantly affect the shoot tissue Ni concentration, however, the combined affect of histidine on biomass production and shoot tissue metal uptake caused a significant reduction in the total quantity of metal removed from solution by plants grown in the Ni + histidine solution compared to plants exposed to the Ni only solution. For flax plants grown in both Cu and histidine, at equimolar concentrations, there was a significant decrease in shoot tissue Cu concentration coupled with an increase in root tissue Cu concentration. These results indicated that the presence of histidine reduced Cu mobility in flax plants by allowing the plant to immobilise the Cu in root tissues possibly as an insoluble complex (Dushenkov *et al.*, 1995). Histidine did not enhance the ability of flax to tolerate either Cu or Ni solutions and reduced the quantity of Cu flax was able to transport to shoot tissue.

The results presented have shown that the plant species most tolerant of both highly and marginally contaminated soils was miscanthus. Miscanthus also removed the greatest quantity of Cd, Cr, Cu, Ni and Zn from the marginally contaminated soils as a result of its' high biomass, whereas flax removed the highest quantity of Pb. Nettle and oilseed rape were identified as the species which had the highest tissue metal concentrations for each of the metals in the marginally contaminated soils. The improvement in nettle and oilseed rape yields observed in the sewage sludge soil compared to the poor yields in artificial soils indicated that these plant species may have potential for improved growth in contaminated soils using soil amendments, in particular fertiliser treatments. These finding suggest that oilseed rape and nettle may play a useful role as phytoremediators under favourable soil conditions.

Cultivation may be a hazard during preparation of a seedbed for any annual crop proposed for phytoremediation such as flax or oilseed rape. The rhizomatous nature of miscanthus and nettle allows the plants to regenerate in successive growing seasons without the need for annual sowing. This has the two-fold advantage of significant cost reductions in agronomy, and of reduced disturbance and possible dispersal of pollutants during cultivation. Miscanthus and nettle, therefore, may be the most appropriate crops for further investigation. Miscanthus which has high biomass and a tolerance to metal-containing soils may be appropriate for chelate-assisted phytoremediation. Nettle which has shown potential to accumulate high tissue metal concentrations could prove successful in continuous phytoextraction if higher yields can be achieved through soil improvements.

This work has given insight into four plant species as potential phytoremediator crops, adding to the growing body of knowledge in the field of phytoremediation. The data presented indicated that herbaceous non-accumulator crops have the potential to tolerate metal contaminated soils and sequester metal in their tissues. Further work based on these data may provide future phytoremediation strategies.

Phytoremediation has been proposed as a potential solution to the problems of contaminated land. Whether or not phytoremediation is an appropriate technique for remediating a particular site will be dependent upon the objectives for the site and resources available. Where sites are highly contaminated and the time scale for remediation is limited, phytoremediation will not be an appropriate strategy. Sites which have a more marginal contamination level and where time scale is less important than the cost of clean up, phytoremediation will be a remediation option. This work has demonstrated that flax, miscanthus, nettle and oilseed rape have the potential to play a role in the remediation of land contaminated by heavy metals. On the basis of the work presented here, firm recommendations on the use of these plants in commercial remediation programmes cannot be given. The results presented in this thesis indicate that herbaceous crops producing moderate to high quantities of biomass such as miscanthus and nettle are likely to be best suited to a mixed cropping remediation strategy incorporating soil amendments to promote biomass yield and metal mobility in the soil-plant system. Resulting biomass will not only have value as a renewable energy feedstock, but in the process of energy production, the concentration and potential recovery of metals from the ash may be possible. Higher value industrial end uses of plant biomass, such as fibres for automotive interiors will also be possible, however, the fate of metal will have to be determined for each use. Further work into these crops in research-based remediation programmes is required in order to establish the merit of their use in commercial remediation strategies.

8 References

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