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SCIENCE DIRECT
Environment International 29 (2003) 529–540
ENVIRONMENT INTERNATIONAL
www.elsevier.com/locate/envint

Review article

Phytoremediation of heavy metal-contaminated land by trees—a review

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Received 20 August 2002; accepted 18 November 2002

Abstract

This paper reviews the potential for using trees for the phytoremediation of heavy metal-contaminated land. It considers the following aspects: metal tolerance in trees, heavy metal uptake by trees grown on contaminated substrates, heavy metal compartmentalisation within trees, phytoremediation using trees and the phytoremediation potential of willow (*Salix* spp.).

Keywords: Contaminated land; Heavy metals; Phytoremediation; *Salix*; Trees; Willow

1. Introduction

Current practice for remediating heavy metal-contaminated soils relies heavily on 'dig-and-dump' or encapsulation, neither of which addresses the issue of decontamination of the soil. Immobilisation or extraction by physicochemical techniques can be expensive and is often appropriate only for small areas where rapid, complete decontamination is required (Martin and Bardos, 1996; BIO-WISE, 2000). Some methods, such as soil washing, have an adverse effect on biological activity, soil structure and fertility, and some require significant engineering costs. Consequently, the low-technology, in situ approach of phytoremediation is attractive as it offers site restoration, partial decontamination, maintenance of the biological activity and physical structure of soils, and is potentially cheap, visually unobtrusive, and there is the possibility of biorecovery of metals (Baker et al., 1991, 1994).

Phytoremediation is defined as the use of plants to remove pollutants from the environment or to render them harmless (Salt et al., 1998). Five main subgroups of phytoremediation have been identified:

- Phytoextraction: plants remove metals from the soil and concentrate them in the harvestable parts of plants (Kumar et al., 1995).
- Phytodegradation: plants and associated microbes degrade organic pollutants (Burken and Schnoor, 1997).
- Rhizofiltration: plant roots absorb metals from waste streams (Dushenkov et al., 1995).
- Phytostabilisation: plants reduce the mobility and bioavailability of pollutants in the environment either by immobilisation or by prevention of migration (Vangronsveld et al., 1995; Smith and Bradshaw, 1972).
- Phytovolatilisation: volatilisation of pollutants into the atmosphere via plants (Burken and Schnoor, 1999; Bañuelos et al., 1997).

The development of phytoremediation is being driven primarily by the high cost of many other soil remediation methods, as well as a desire to use a 'green', sustainable process.

Initially, much interest focused on hyperaccumulator plants capable of accumulating potentially phytotoxic elements to concentrations more than 100 times than those found in nonaccumulators (Salt et al., 1998; Chaney et al., 1997; Raskin and Ensley, 2000). These plants have strongly expressed metal sequestration mechanisms and, sometimes, greater internal requirements for specific metals (Shen et al., 1997). Some species may be capable of mobilising metals from less-soluble soil fractions in comparison to nonhyperaccumulating species (McGrath et al., 1997). Metal concentrations in the shoots of hyperaccumulators normally exceed those in the roots, and it has been suggested that metal hyperaccumulation has the ecological role of providing protection against fungal and insect attack (Chaney et al.,

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0160-4120/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0160-4120(02)00152-6

1997). Such plants are endemic to areas of natural mineralisation and mine spoils (Brooks, 1998). Examples include species of *Thlaspi* (Brassicaceae), which can accumulate more than 3% Zn, 0.5% Pb and 0.1% Cd in their shoots (Baker et al., 1991; Brown et al., 1994), and *Alyssum* (Brassicaceae), some species of which have been shown to accumulate over 1% Ni (Brooks et al., 1979).

Exploitation of metal uptake into plant biomass as a method of soil decontamination is limited by plant productivity and the concentrations of metals achieved (Baker et al., 1991). For instance, *Thlaspi caerulescens* is a known Zn hyperaccumulator, but its use in the field is limited because individual plants are very small and slow growing (Ebbs and Kochian, 1997). The ideal plant species to remediate a heavy metal-contaminated soil would be a high biomass producing crop that can both tolerate and accumulate the contaminants of interest (Ebbs and Kochian, 1997). Such a combination may not be possible, there may have to be a trade-off between hyperaccumulation and lower biomass, and vice versa. Furthermore, the cropping of contaminated land with hyperaccumulating plants may result in a potentially hazardous biomass (Bañuelos and Ajwa, 1999).

An alternative to the use of hyperaccumulators is the use of nonaccumulator plants, possibly coupled with manipulation of soil conditions either to increase the bioavailability and, hence, increase plant uptake, or the stabilisation, and so decrease plant uptake, of metals. For example, there are two major limitations to Pb phytoextraction: the low Pb bioavailability in soil and the poor translocation of Pb from roots to shoots. Huang et al. (1997) investigated the potential of adding chelates to Pb-contaminated soils to increase Pb accumulation in plants and showed that concentrations of lead in corn and pea shoots were greatly increased. Ethylenediaminetetraacetic acid (EDTA) was the most effective chelate in increasing Pb desorption from soil into the soil solution and also greatly increased the translocation of Pb from roots to shoots through prevention of cell wall retention. There is, however, the possibility that EDTA added to soil may mobilise heavy metals that can then be leached into the subsoil or into ground- or surface waters and measures to prevent metal leaching, such as application of chelate solutions to meet plant water needs and tile drains to capture leachate, may be necessary (Cooper et al., 1999). Vangronsveld et al. (1995, 1996) used beringite, a waste product from the burning of coal refuse, to immobilise heavy metals in a contaminated soil, thereby decreasing their phytotoxic effects.

Salt et al. (1998) noted the potential of manipulating metal resistance mechanisms in nonhyperaccumulating plants to improve phytoextraction. This could be done by conventional plant breeding programmes or by genetic manipulation. However, improved metal resistance alone may not be sufficient for successful phytoextraction, which also depends on metal bioavailability, root uptake and shoot accumulation.

As a result of the concerns regarding use of hyperaccumulators, enhanced remediation using chelates and genetic manipulation of plant traits, there has been considerable interest recently in the potential use of trees for phytoremediation. They are high biomass producers and for certain species, such as *Salix*, the tremendous genetic variability is already being exploited through plant breeding programmes (Larsson, 1994; Lindegaard and Barker, 1997).

2. Metal tolerance in trees

The long generation time of trees acts to prevent a rapid selection of heavy metal tolerant genotypes, the production of which is random or induced by the pollutant (Dickinson et al., 1991). Therefore, tree species are generally not able to adapt to high concentrations of heavy metals in the soil, resulting in the evolution of only a few metal-tolerant ecotypes (Kahle, 1993). A characteristic feature of metalliferous soils in Europe is the absence of woody and tree species (Turner, 1994). However, the lack of reported toxicity symptoms in trees indicates that their tolerance mechanisms may allow them to withstand higher heavy metal concentrations than agricultural crops (Riddell-Black, 1993). Trees that are not especially selected for metal tolerance can generally survive in metal-contaminated soil, albeit usually with a much reduced growth rate (Dickinson et al., 1992).

Restricted location of metals in roots and low uptake into foliage is the most common resistance trait (Dickinson and Lepp, 1997), but true tolerance requires the development of one or more precise physiological mechanisms with a genetic basis (Dickinson et al., 1991). But the genetic stability of tolerance is questionable, as it can be both induced and lost in trees, so their ability to acclimatise to fluctuating stress due to pollution may be very important for the survival of tree species. Dickinson et al. (1992) described tolerance and survival of plants on metal-contaminated soils as arising from "an orchestrated multiplicity of physiological and biochemical responses, including both avoidance and true resistance mechanisms".

The wide genome of trees and facultative tolerance, such as the redistribution of roots to less contaminated zones of soil, allows survival of trees not selected for metal tolerance on polluted soils. Various studies have shown that the acclimation of trees to their soil environment is of considerable importance and may be a significant factor in tree survival in metal-contaminated soil, although soil fertility can be important and mycorrhizal fungi, the hyphae of which can sequester metal ions, may increase metal resistance.

Borgegård and Rydin (1989) considered the effects of heavy metals on root development in birch trees which had colonised soil covering copper mine tailings. Tree survival was good despite roots penetrating into the spoil; an example of facultative tolerance in a species, not especially selected for metal tolerance, but also not being excluded from a substrate containing high heavy metal concentrations. Kopponen et al. (2001) tested the tolerance to Cu and Zn in birch clones from metal-contaminated sites when grown in an artificially contaminated soil. They found considerable variation in the tolerance of samples from within the same population. There was, however, an overall pattern in which tolerance was related to the type of contamination at the site from which the clones were sampled, i.e. clones collected from a copper smelter site grew better in Cu-contaminated soil than clones from a Zn smelter site, and vice versa. Experiments comparing the growth of tree seedlings from trees in metal-polluted and uncontaminated areas failed to demonstrate the existence of tolerance traits (Dickinson et al., 1991), implying that the adaptation of individual mature plants may be the most significant factor which determines the ability to survive pollution. Turner and Dickinson (1993) also showed this. Growth of sycamore seedlings collected at metal-contaminated and control sites, were compared in both metal-amended nutrient solutions and reciprocal transplant experiments in soils. Even in contaminated soils, most seedlings survived for at least 3 years despite impaired growth, suggesting that some low level of innate tolerance may exist, but that facultative tolerance, such as the observed proliferation of fine roots in uncontaminated zones of the soil, is very important. Watmough and Dickinson (1995) summarised this as a strategy of avoiding toxicity, removing selection pressure and negating the necessity for the evolution of tolerance.

Acclimation of trees to metal stress has been studied using various indices such as cell suspension cultures (Dickinson et al., 1992) and callus cultures (Watmough and Dickinson, 1995), but seedling growth is the most commonly used index, despite their greater sensitivity to adverse conditions than mature trees. Turner and Dickinson (1993) questioned whether studies on seedlings accurately reflect the responses of older trees. Mature trees differ from seedlings in a number of ways, including carbon allocation, canopy structure and the fraction of photosynthetically inactive tissue, which increases with age (Turner, 1994). Seedlings are much more sensitive to adverse conditions, leading to false indications of the accumulation capacities of mature plants (Riddell-Black, 1993). Nevertheless, the response of seedlings or cuttings to heavy metal exposure remains the most common way of assessing the ability of different species, or clones of the same species, to take up, tolerate and survive such stress. Screening 16 willow clones for resistance to copper in solution culture, Punshon et al. (1995) found differences in metal uptake patterns and root production. Considerable variability in copper resistance was recorded between willow species and hybrids. The degree to which observed resistance was genetically determined or induced by the environment was unclear. In further work, Punshon and Dickinson (1997) showed that resistance to heavy metals in willow could be increased by gradual acclimation of the trees to metal stress. Landberg and Greger (1994) tested 94 clones of *Salix viminalis* and *Salix dasyclados* for tolerance to heavy metals using a hydroponic system. They showed considerable variation in tolerance and metal uptake between clones of these two species. For example, the Cd concentration in the shoots of clones exposed to 1 μM Cd solution for 20 days differed by a factor of 80 between the lowest and highest concentrations. Uptake and metal accumulation were not related to tolerance, and there was evidence of both general tolerance to a number of different metals and specific tolerance to one heavy metal. They stressed the point that for the purposes of phytoremediation, not only the accumulation capacity but also biomass production must be considered (i.e. the amount of heavy metal taken up from soil per hectare). Watson et al. (1999) developed a nutrient thin film hydroponic technique (NFT) that could be used for rapid screening of tree cuttings for tolerance to heavy metals based on biomass production in condition of metal exposure. A test on 15 *Salix* clones showed that there was good agreement between results from the NFT screen and the biomass production and metal uptake by the same clones grown in a field trial (Watson, 2002; Pulford et al., 2002).

3. Heavy metal uptake by trees grown on contaminated substrates

Bioavailability of metals to trees and subsequent metal accumulation in tree tissues can vary hugely according to the source of metal contamination and site conditions. Lepp and Eardley (1978) examined the effects of metal-contaminated sludge on the growth and metal content of sycamore seedlings over 50 days. The beneficial effects of sludge on tree growth far outweighed any impact of the sludge-borne metals on growth processes over the duration of the experiment. Sludge application effectively raised the pH of the growing medium, resulting in suboptimal conditions for uptake of Pb, Zn and Cu, so metal burdens were not excessive and showed no significant relationship to soil metal concentrations. Similarly, Labrecque et al. (1995) found the amounts of metals added to soil by sludge did not induce phytotoxicity symptoms or decrease biomass in two willow species. Hasselgren (1999) found stem biomass production of three willow clones was enhanced by sludge application rate; it also led to more uniform growth and a greater shoot number than in control plots. Concentrations of metals in plant tissues were not influenced by application, but generally decreased with stem and stand age. Berry (1985) grew loblolly pine seedlings in plots amended with five different sewage sludges and monitored the growth and heavy metal accumulation of the trees. For some sludges, toxicity of heavy metals to the trees was considered to be a factor in poor seedling growth relative to an inorganic fertiliser control, although sludge application rate did not affect seed germination or seedling survival. Cadmium and

Zn concentrations in the foliage generally varied with the concentrations in a particular sludge. Cadmium and Zn uptake was calculated to be about 1% of the amount applied, a degree of uptake not large enough to significantly decontaminate the soil.

Analysing heavy metal concentrations in various trees (maple, ash, pine, birch and cottonwood) grown on sludge-amended mine spoil, Morin (1981) found first year tree samples indicated rather high metal accumulation patterns within the tree species; third year harvest samples did not exhibit any significant increases in concentrations. This did not necessarily mean a decline in metal assimilation over time. It was attributed to dilution by higher biomass production and accumulation of metals in the litter layer. Korcak (1989) assessed the Cd uptake of various fruit trees under two pH regimes. Soil treatments that significantly raised soil pH caused lower Cd concentrations in pear tree bark and wood compared to low pH, which generally produced slightly higher leaf and root Cd. Uptake of Cu, Zn and Ni by trees planted on slag at the Lanarkshire Steelworks in Scotland was found to be less than 1% of the metal contained in the top 10 cm of the waste, rendering phytoextraction unfeasible at this site (Salt et al., 1996). The low uptake of metals was attributed to the high pH of the waste and the chemical form of the slag's metal contaminants. Borgegård and Rydin (1989) analysed tissue concentrations of heavy metals in birch trees that had colonised soil covering copper mine tailings. They found leaf concentrations of Zn, Pb and Cd exceeded values for leaf concentrations of trees growing in uncontaminated soils by about one order of magnitude, whereas Cu concentrations were similar. Fernandes and Henriques (1989) compared the Zn, Cu and Pb concentrations in leaves and fruits of holm-oak trees at the outskirts of a pyrites mining area with those in suitable controls. The trees growing in substrate affected by the mining showed pronounced stunting, reduced leaf size and extensive necrotic and chlorotic spotting, and had concentrations more than 50 times higher for Cu, and 20 times higher for Pb and Zn. Sampling stems and leaves of *Salix*, *Silene* and *Populus* species growing on mine spoil, Dinelli and Lombini (1996) observed that metal concentrations were generally higher in the early vegetative growth stage, due to a relatively high nutrient uptake compared to growth rate. This was followed by a period of vigorous growth, which diluted the concentrations until the flowering stage, in which the minimum values for almost all elements were obtained. Senescence of trees usually produced an increase in metal levels due to concentration caused by loss of fluids.

Seasonal variations of the foliar metal concentrations in woody plants have been confirmed by other studies. A 2-year study of the metal content of birch leaves by Ehlin (1982) showed that Cu and Ni contents decreased at the beginning of the growth period, probably a dilution effect resulting from increased dry weight of the leaves. A marked accumulation of Zn in sycamore, beech, horse chestnut and hazel leaves at the end of the growing season has been noted by Ross (1994). This was interpreted as metal shunting occurring in the plant tissues prior to senescence, or seasonal variation in soil metal availability. Riddell-Black (1994) reported consistent increases in foliar heavy metal concentrations shortly before senescence in willow grown on a metal-contaminated substrate. Hasselgren (1999) interpreted a tendency of increased willow leaf Cu content in autumn as a possible detoxification effect in connection with defoliation. At a less-contaminated site, Watson (2002) reported a decrease at the end of the growing season in the concentrations of Cd, Cu and Zn in leaves and bark, but a slight increase in concentrations in wood.

4. Heavy metal compartmentalisation within trees

Trees differ in their ability to translocate heavy metals from the root to the shoot. In sycamore seedlings grown in sludge-amended soil, Lepp and Eardley (1978) found concentrations of metals in the stems and leaves to be an order of magnitude less than corresponding root levels. Morin (1981) found root tissues of several tree species grown on sludge-amended spoil had the highest concentrations of Cd, Cu, Ni and Zn. Turner and Dickinson (1993) found, in sycamore trees grown in contaminated soil, most of the Pb not retained in the roots was translocated to the stem, while most of the Zn not retained in the roots was translocated to the leaves. McGregor et al. (1996) analysed tissue of sycamore, birch and willow trees which had naturally established on sites contaminated by waste from an explosives factory and a chromium processing works. Chromium, Pb and Cu were found to have accumulated mainly in the tree roots; Zn concentrations were highest in bark. Numerous studies have shown accumulation to occur in actively growing tissues such as shoots and young leaves: Drew et al. (1987) grew poplar clones in sludge-amended soil and found Zn and Cd concentrations to be the highest in foliage. In four willow species grown on sludge-amended soil, foliage concentrations were greater than those in the stem for all varieties and metals (Riddell-Black, 1994). Of the above-ground biomass metal concentrations determined in sludge-amended willow plots by Hasselgren (1999), Cu, Pb and Cr were mainly in the stems, while Zn, Cd and Ni were in the leaves.

Partitioning of the metals within the stem can vary within a tree. Punshon et al. (1996) found some willow clones compartmentalised metals, especially Cd, in woody tissues much more markedly than others. Pulford et al. (2002) classified 20 willow varieties grown on sludge-amended soil into two groups: those which accumulated Cu and Ni in above ground biomass (which suffered reduced yield) and those which did not. Bark concentrations of all heavy metals determined were found to be consistently greater than the concentrations in wood of the same clone. Korcak (1989), however, reported little difference between bark and wood

Cd concentrations in several fruit tree species. Within the stem, elements can be radially transported. Rapid lateral movement of water from xylem to phloem in 3-year-old *Salix* trees has been demonstrated by Epstein (1972), while Hagemeyer and Hubner (1999) reported a conceivable redistribution of Pb in stems of spruce trees, possibly via the axial xylem sap stream or in rays.

Concentrations of Cu and Zn were found to vary between tree compartments in eight *Salix* species studied by Nissen and Lepp (1997). The species did not show a common uptake pattern for the two metals. A general trend of exclusion of Cu and concentration of Zn in shoot tissue relative to soil concentrations, however, was evident. The low concentrations of Cu reported point to exclusion from the shoot system, reflecting the low mobility of Cu following root or foliar uptake. Considerably higher leaf and bark concentrations of Zn reflected the mobility of the element after uptake, whereas low concentrations in wood displayed a low retention of Zn in the xylem tissues. It was acknowledged that the partitioning of metals between tissues may change as the soil metal levels increase.

Sander and Ericsson (1998) found concentrations of Zn, Cu, Ni and Cd in stems of *Salix viminalis* increased significantly with height, thought principally to be a consequence of increasing bark proportions. As the stem narrows towards the shoot top, the proportion of bark increases, which generally contains a greater concentration of plant nutrients than wood. Nickel increased most from the lowest to the highest sampling level, followed by Cu, Zn and Cd. Different elemental reallocation patterns during leaf senescence may have led to the different concentration gradients in the shoots after leaf fall. The effect of nutrient availability on the allocation patterns of the elements was also questioned.

Wood and bark are important sinks for biologically available metals, with additional sink tissue being formed each growing season. These tissues are slow to enter the decomposition cycle; accumulated metals can, therefore, be immobilised in a metabolically inactive compartment for a considerable period of time (Lepp, 1996), if the contaminated trees are not reused for other purposes which accelerate the return of the heavy metals to the environment, such as in combustion. While metal concentrations in wood are frequently lower than in roots and bark, the fraction may represent a much more significant proportion of the total amount of metal in a tree (Dickinson and Lepp, 1997).

5. Phytoremediation using trees

The potential use of trees as a suitable vegetation cover for heavy metal-contaminated land has received increasing attention over the last 10 years (Aronsson and Perttu, 1994; Glimerveen, 1996; EPA, 1999, 2000). Trees have been suggested as a low-cost, sustainable and ecologically sound solution to the remediation of heavy metal-contaminated land (Dickinson, 2000), especially when it is uneconomic to use other treatments or there is no time pressure on the reuse of the land (Riddell-Black, 1994). Benefits can arise mainly from stabilisation of the soil or waste, although in some cases phytoextraction may be sufficient to provide clean up of the soil. Before these benefits can be realised, the trees must become established on a site.

On highly contaminated soils, or on mining wastes, tree establishment may be inhibited by high concentrations of heavy metals. Under such conditions root immobilisation, which would normally protect a plant, may not be able to prevent toxic amounts of metal being translocated to the aerial parts of the plant. In less-contaminated soils, other factors may limit plant growth; such as macronutrient deficiencies (Pulford, 1991) and physical conditions, especially those properties leading to poor waterholding, aeration and root penetration (Mullins, 1991).

The physical and hydraulic conditions of a site are of primary importance to tree establishment. Mine spoil heaps are not homogenous in composition or permeability. For example, layers of anomalously fine material containing metal concentrations up to 100 times greater than those in bulk tailings have been observed at historic metalliferous mine sites in the UK (Merrington, 1995). Therefore, the vertical and horizontal extent of the contamination, properties affecting rooting and nutrient distribution can vary considerably in the rooting substrate, and this heterogeneity can result in wide variations in tree survival. Bending and Moffat (1999) carried out a study of the relationship of tree growth with the chemical, physical, nutritional and hydrological properties of minespoil. Establishing trees on minespoil was affected by compaction, infertility (especially N deficiency), acidity, salinity and poor water-holding capacity. Restricted rooting occurred as a result of a shallow water table and high bulk density of the substrate. Many tree species can grow on land of marginal quality in terms of fertility, texture and structure. Jobling and Stevens (1980) reported that a number of pioneer tree species, including several willow varieties, had high survival and growth rates in a variety of substrate and climatic conditions. Taking saplings or cuttings of selected clones of birch and willow from spoil heaps and testing growth on restored opencast coal sites, Good et al. (1985) found many achieved consistently higher mean survival than unselected controls over the range of sites. Generally, willow achieved higher shoot growth increments than birch, and the greatest gains in survival occurred on the poorest growth medium (where topsoil was not used), whereas there was little advantage in their use on fertile sites with good drainage. It was recognised that a low growth rate, with high survival, on these nutrient deficient sites is preferable to low survival coupled with a continued fertiliser application requirement to sustain growth. While the addition of organic amendments such as sewage sludge may aid revegetation, roots may not extend readily from a fertile layer into underlying contaminated material, and it may increase the weed problem in some young woodland areas. Johnson et al. (1977) described cessation of root development at the interface of Pb/Zn spoil and organic amendments.

Phytostabilisation can result from either physical or chemical effects. Once the trees have become established, the vegetation cover can promote physical stabilisation of a substrate, especially on sloping ground. Long-term stability of the land surface can be achieved as the standing trees decrease erosion of the substrate by wind and water (Johnson et al., 1992). Trees have massive root systems, which help to bind the soil (Stomp et al., 1993), and the addition of litter to the surface quickly leads to an organic cover over the contaminated soil. In addition, transpiration of water by the trees reduces the overall flow of water down through the soil, thus, helping to reduce the amounts of heavy metals that are transferred to ground- and surface waters. Garten (1999) modelled the effect of a forest cover on the loss of ^{90}Sr by leaching from contaminated soil, mainly in shallow subsurface flow, and showed that such losses were reduced by approximately 16% under trees relative to grass. This was attributed to the greater rate of evapotranspiration by the trees.

Phytostabilisation of a heavy metal-contaminated substrate may also be achieved by causing chemical changes to specific metals, which result in their becoming less bioavailable. Chaney et al. (1997) identified two elements, Cr and Pb, which may be immobilised by a vegetation cover. They suggested that deep rooting plants could reduce the highly toxic Cr(VI) to Cr(III), which is much less soluble and, therefore, less bioavailable (James, 2001). Although no mechanism for this was suggested, organic products of root metabolism, or resulting from the accumulation of organic matter, could act as reducing agents. It is known that Cr tends to be held in plant roots, whether supplied as Cr(VI) or Cr(III) (Pulford et al., 2001), which may also suggest reduction and immobilisation in the roots. Lead may be immobilised by the formation of the lead phosphate mineral chloropyromorphite in soils and within roots (Cotter-Howells et al., 1994), which has been shown to be formed in soils by *Agrostis capillaris* growing on lead/zinc mining wastes (Cotter-Howells and Caporn, 1996).

The effect of vegetation on the bioavailability of other metals is uncertain. Pulford et al. (2002) showed that the concentrations of EDTA extractable Cd, Cu, Ni and Zn in sewage sludge treated soil were higher under willow than in unplanted areas. On the other hand, Watson (2002) found evidence for depletion of extractable metals due to biomass willow growth, when compared to concentrations in adjacent unplanted areas. Plant concentrations accounted for only a small fraction of the observed depletions due to plant uptake; therefore, redistribution of metals amongst the soil solid phases was considered likely. Sequential extractions of metals from soils demonstrated this: where trees were growing, or had been recently harvested, extractable concentrations frequently fell significantly in the period from March to June, with a corresponding rise in the residual fraction. Conversely, soil solution concentrations were significantly higher in soils affected by root activity than in unplanted control soils. This is likely to be due to increased complexation of heavy metals by soluble chelating agents exuded by plant roots or root respiration. Cutting of the willow significantly increased the concentrations of Pb in soil solution six months after tree harvest, which was interpreted as an effect of root degradation (Watson, 2002). The effect of plant roots on the chemistry and bioavailability of heavy metals in contaminated soils is an area that requires much more study in order to assess the potential of trees to immobilise or release the metals.

For the purposes of phytoextraction, Punshon et al. (1996) suggested that the following characteristics were beneficial:

- ability to grow on nutrient-poor soil
- deep root system
- fast rate of growth
- metal-resistance trait

In addition, an economically viable secondary use would be desirable. Trees have been shown to meet all of these requirements, the first three in particular. While a high metal content in agricultural crops is not desirable, and indeed is potentially dangerous, a higher metal content in trees is acceptable, as long as normal physiological activity is not affected (Labrecque et al., 1995). While no commercially important trees are known to hyperaccumulate metals (Riddell-Black, 1993), heavy metal availability to trees can be increased relative to availability to nonwoody plants through increased wet and dry deposition to leaf surfaces and soil, altered cuticular surface characteristics leading to greater availability of foliar-deposited metals, and soil acidification induced by tree growth causing increased metal mobilisation in soils.

Dickinson (2000) reported several factors besides heavy metals to be important in the performance of trees grown on metal-contaminated sites. Weed competition and subsequent neglect may influence survival (Rees et al., 1998), and inadequate soils (leading to poor fertility and/or water shortage) can also have a major effect. Cultivation will aerate the soil and promote the activities of soil microbes and, hence, the breakdown of organic matter. Besides improving amenity, the trees provided habitats for wildlife and improved prospects of soil formation due to a build-up of litter following leaf-fall.

Studies of tree establishment on contaminated land have considered a number of different species, e.g. *Salix* (Willow), *Betula* (Birch), *Populus* (Poplar), *Alnus* (Alder) and *Acer* (Sycamore). While many of these studies were interested primarily in metal uptake, distribution within the plant and tolerance mechanisms, for the purposes of phytoremediation, most attention has been paid to fast growing species, such as willow.

6. The phytoremediation potential of willow (*Salix* spp.)

The genus *Salix* is a member of the *Salicaceae* plant family. There are 400 species of willow, with more than 200 listed hybrids (Newsholme, 1992). The majority of the genus *Salix* grow in lowland wetland habitats and have evolved a number of varieties and hybrids (Sommerville, 1992). The large number of species and hybrids of *Salix* suggest a wide genetic variability within the genus. While there are creeping forms such as *Salix repens* and willow bushes (for example *Salix aurita*), most species are multi-stemmed small trees such as *Salix caprea* and *Salix cinerea*. A few species are single trunk trees which reach to over 20 m in height, such as *Salix alba*. In almost all, falling trunks or branches touching the ground can take root, and shoots grow rigorously from a coppiced stool (Sommerville, 1992).

The genus features many species of high productivity and invasive growth strategies (Punshon et al., 1996). Many species, such as *S. caprea* and *S. cinerea*, and the hybrid *S. viminalis*, are known to colonise edaphically extreme soils (Dickinson et al., 1994). Mang (1992) detailed the importance of willow in colonising heavy metal-contaminated dried silt removed from a port, and drew attention to the suitability of selected clones for planting in polluted areas. The soil consolidation provided by the enmeshment of their spreading roots is a feature that can be exploited in the reclamation of land. The demand of the perennial root system of willows for water lowers the risk of contaminant leaching (Sander and Ericsson, 1998), and most species are able to tolerate long drought periods.

A characteristic of willow, which makes it a very suitable tree for use in phytoremediation, is that it can be frequently harvested by coppicing, yielding as much as 10–15 dry t ha^{-1} $year^{-1}$ (Riddell-Black, 1993). Bushy *Salix* species with erect stems, rapid growth and good rooting ability are the most suitable for biomass coppice, with *S. viminalis* being one of the most widely used species (Ahman and Larsson, 1994). In addition to high biomass productivity, *Salix* trees also have an effective nutrient uptake, high evapotranspiration rate and a pronounced clone specific capacity for heavy metal uptake.

Possible end-product uses of *Salix* biomass include fuel for direct burning as wood chips, raw material for the production of paper, chipboard and charcoal, a source of viscose for the textile industry, basket weaving and the production of briquettes, ethanol and ruminant livestock feed supplement (McElroy and Dawson, 1986). Use as wood fuel could allow possible heavy metal recovery through the scrubbing of smoke gases and proper handling of ashes (Perttu and Kowalik, 1997; Dahl, 2000).

Two possible strategies have been proposed for the use of willow for phytoremediation (Punshon et al., 1995).

- Willows that survive in contaminated soil with minimal uptake of metals into the aerial tissues would be most appropriate for use where distribution of heavy metals to the wider environment or transfer of metals into the food chain is to be avoided.
- Willows that accumulate relatively high amounts of metal are desirable if soil remediation is to be achieved by phytoextraction and tree harvesting.

Various approaches have been taken to study the uptake of metal by willow clones involving field trials, glasshouse pot experiments and hydroponic systems. Landberg and Greger (1994) tested various clones for Cd and Zn accumulation and tolerance in solution culture. Some clones were tolerant to both metals, others to just one; tolerant clones could have a relatively high or low net uptake of metal, with the net transport to the shoots varying between 1% and 72% of the total metal uptake. Greger (1999) found Cd uptake capacity of 70 *Salix* genotypes could differ by as much as 43 times between the clones with the highest and lowest values. The better varieties had capacities about five times higher than the hyperaccumulators *T. caerulescens* and *Alyssum murale* due to high biomass production and transport of Cd to the shoot. Felix (1997) reported *Salix viminalis* had the highest metal-accumulating ability of the various plants tested: it achieved a transfer coefficient of 3.4 for Cd in a field trial on a contaminated soil. However, the calculated 77 years to decontaminate the soil used in this study to acceptable Cd concentrations were not practicable, highlighting the limitations of yields and/or metal uptake rates to phytoextraction as a remediation tool.

Riddell-Black (1993) calculated the stem concentrations of metal which would have to be achieved to reduce metal concentrations in a polluted soil to target concentrations over 30 years (the expected productive life of willows grown on short rotation). It was shown that the decontamination of highly contaminated land could not be achieved in the short term due to the very high stem concentrations of metals required. Remediation of less polluted land may be achievable in a reasonable timescale, even with low metal uptake, due to the frequency of harvest. The success of *Salix* as a phytoextracting plant depends on its biomass production, metal accumulation capacity and the site of metal accumulation in the plant (Riddell-Black, 1994). Uptake of heavy metals by four varieties of *Salix* used in woodchip production for energy was measured. The trees were grown for 3 years on soil that had received sewage sludge for over 50 years. The main benefit from *Salix* growth on this metal-contaminated site was reported to be site stabilisation, as metal uptake by the harvested biomass was not sufficient for phytoextraction to be realistic. However, she stated the growth of *Salix* may be useful if depletion of the bioavailable metal in a soil occurs.

There is encouraging evidence of this occurring from other studies. About 30% of the bioavailable Cd was removed by *Salix* in a 90-day pot trial (Greger, 1999). Eriksson and Ledin (1999) found concentrations of exchangeable Cd in 8 soils taken from *Salix* stands and nearby unplanted reference soils were 30–40% lower in the planted soils. Total Cd concentrations were not significantly reduced, but the exchangeable Cd pool was reduced. Uptake occurred throughout the soil profile to a depth of 65 cm. However, Alriksson et al. (1999), investigating the effects of the growth of five tree species (including willow) over 6 years on Cd soil concentrations, found high biomass Cd uptake was not related to a corresponding depletion of the soluble Cd pool. This pool increased due to decreasing pH.

The bark and wood concentrations of heavy metals in 20 willow varieties were determined by Pulford et al. (2002). Overall, the concentrations in the 3-year-old trees suggested certain clones have potential to take up significant quantities of metals. Stem Cd concentrations in this study were up to an order of magnitude greater than soil concentrations. Punshon et al. (1995) also found various *Salix* clones compartmentalised Cd in woody tissues much more markedly than others, representing a beneficial trait for long-term removal of the contaminant from the soil. Ostman (1994) calculated the annual Cd uptake by willow (exceeding that supplied by fertilisers and air deposition) to be around 3–4% of the plant available Cd in Swedish soils, and suggested that a rotation cycle of around 20–25 years would reduce the Cd concentrations to below natural levels. Commercially grown *Salix* has been shown to accumulate 20–30 g Cd per hectare per year (Goransson and Philippot, 1994).

A number of studies have supported this observation that Cd uptake by willow from moderately contaminated soil may be sufficient to allow clean up within a few years. As only one hyperaccumulator of Cd, *T. caerulescens* (Brooks, 1998), is known, there is considerable potential to exploit this ability of willow to take up large amounts of Cd. Cadmium is highly zootoxic and is a common contaminant in the urban environment. Thus, the use of willow to remove Cd from moderately contaminated soil may be the most immediate practical application of phytoremediation.

The general trend of exclusion of Cu and accumulation of Zn in shoot tissue of eight *Salix* species, identified by Nissen and Lepp (1997), indicates a low potential for the depletion of soil Cu through repeated harvests, whereas there is some potential for Zn. Dickinson et al. (1994) carried out a series of experiments to investigate the establishment and growth of a number of *Salix* clones placed in metal-contaminated soils, and the uptake and partitioning of the metals within the plant. Translocation from the roots to the shoots was greatest for Zn (stem concentrations were as high as 0.8% in plants grown in mining spoil), making removal of the metal during harvest feasible. McGregor et al. (1996) noted that changes in Zn concentrations within tree tissue in different parts of the growing season suggested an optimum harvest time would be during winter, fitting in well with usual coppicing practice. However, calculation of the time required to reduce the zinc concentration in the soil to acceptable levels suggest that the timescale is too long (approximately 800 years in this case).

Labrecque et al. (1994) estimated the bioaccumulation of various metals by *Salix discolor* and *S. viminalis* with respect to metal application in sludge. About 50–80% of the total quantity of bioaccumulated metals were found in the roots and stem–branch biomass, representing an immobilization of metals relative to that accumulated in leaves, which is returned to the soil at the end of each growing season. The metal transfer coefficients for Cd and Zn were considerably greater than those for Ni, Cu and Pb (Labrecque et al., 1995). Increased metal application to the trees did not necessarily lead to increased tissue metal concentrations; Cu, Ni and Pb plant concentrations were less dependent on soil concentrations, whereas those for Cd and Zn were more so, pointing to higher soil solution solubilities of these metals and species preference for them.

Landberg and Greger (1996) gauged the tolerance and accumulation of Cd, Cu and Zn of stems of different *Salix* clones grown on metal polluted and unpolluted areas. They found no differences between the polluted and control areas in the tolerance of *Salix* to the metals or in concentrations of the heavy metals in the collected stems. However, growth of clones from the polluted area was generally stimulated at a low metal concentration. The variation in accumulation and tolerance to heavy metals was wider within the species than between the species. Clones from the polluted area had higher metal accumulations in their roots, and a lower translocation of metals to the shoots, probably a mechanism to protect the shoot.

Punshon and Dickinson (1999) investigated the resistance of *Salix* to Zn, Cd, Cu and Ni in hydroponic experiments, and reported considerable variation between and within clones. Resistance was not species-specific, but rather clone- or hybrid-specific. The considerable intraclonal variation was demonstrated by the survival of a proportion of cuttings of some of the most sensitive clones in the high metal regimes. To put this into context, the large variation of the growth of *Salix* in uncontaminated soils was acknowledged. This variation causes difficulties in phytoremediation screening programs, but may be essential in allowing provision of a remediation technique for a site through selective planting.

7. Conclusions

The use of trees as a vegetation cover for the phytoremediation of land contaminated by heavy metals does seem to have considerable potential. There is plenty of evidence from the natural establishment of trees on contaminated sites that some types of trees can survive under such adverse conditions. The rate of growth may be less than would be expected on uncontaminated sites, but that is not particularly important in this case. Survival seems to be due to facultative tolerance, such as avoidance by roots of highly contaminated substrate or by immobilisation of heavy metals in the root. Specific, genetically transmitted tolerance systems have not evolved. There is, however, some evidence that tolerance may be increased by acclimation of individual trees to low concentrations of heavy metals.

Heavy metals have different patterns of behaviour and mobility within a tree. Lead, chromium and copper tend to be immobilised and held primarily in the roots, whereas Cd, Ni and Zn are more easily translocated to the aerial tissues. This has important implications to the control of movement of heavy metals in the wider environment, which is one of the primary aims of phytoremediation. Heavy metals cause problems at high concentrations and when they are sufficiently environmentally mobile that they can move between media (e.g. soil to water) or can be taken up by living organisms. Immobilisation of, for example, Cr in the root zone under a tree cover may be an appropriate and economic way of limiting potential Cr toxicity. On the other hand, uptake of, for example, Cd into the harvestable parts of trees may be a suitable way to clean up contaminated soil. Both approaches have their problems—retention of the pollutant on site in the case of Cr; concentration of the pollutant into plant tissue, which may create a disposal problem, in the case of Cd. These differences in behaviour do, however, allow a different approach to be taken to remediation depending on the main pollutant of concern.

The main characteristic of trees that makes them suitable for phytoremediation is their large biomass, both above and below ground. Physical phytostabilisation can be readily achieved and is often the main benefit of using trees on such sites. In addition to the direct stabilisation of the soil by the tree roots, the vegetation cover decreases the risk of soil loss by wind and water erosion. Leaf fall adds significant amounts of organic matter to the surface layers of the soil, promoting nutrient cycling, soil aggregation and water holding ability. Dead tree roots and root exudates also contribute to this. The large amount of water removed from soil by the transpiration stream decreases the downward flow through the soil, and so reduces leaching losses. Overall, therefore, the growing of trees can contribute positively to physically stabilising contaminated land. The evidence for chemical phytostabilisation is still sparse, and this is an important area requiring research in the future.

There is a lot of interest specifically in the use of willow (*Salix* spp.) for phytoremediation. In particular, the use of fast-growing, bushy species, which can be readily grown under a short rotation coppice system, with harvests every 3–5 years, show considerable promise. The fast growth and regular harvests lead to rapid uptake of nutrients, and hence also heavy metals, from the soil. Burning of the harvested wood to produce renewable bioenergy is also an attractive feature when considering the overall life cycle of the system. The large willow breeding programmes, for example in Sweden and the UK, provide the opportunity to produce clones that have suitable characteristics for phytoremediation—especially high biomass production, high metal uptake and tolerance. So far, the evidence is that short rotation coppice willow may be a suitable system for decontaminating soil with slightly elevated concentrations of Cd—for example, in agricultural land resulting from high rates of application of phosphate fertilisers. Field trials have suggested that clean up of such soils could be achieved within a few years. Similar evidence is not available for other elements, with estimates of hundreds, or even thousands, of years being required to clean up soil contaminated with elements such as Pb and Zn. The question that needs to be addressed here is what measure of metal contamination should be used to assess clean up. The estimates above are made on the basis of total metal content, whereas a measure of bioavailable metal may be more appropriate. There is already some evidence that bioavailable fractions of heavy metals may be decreased by growing willow, but the replenishment rates of such fractions are unclear, as are the effects trees can have on the distribution of metals between pools.

Phytoremediation has many advantageous features that make it an appropriate and successful technology, giving practitioners a valuable option for remediation. Its major advantage is the low cost, estimated by the USEPA to be 50–80% lower than the alternatives for some applications of phytoremediation (EPA, 2000). In most cases, engineering costs are minimal and this, along with the effects of a vegetation cover, helps limit the spread of contamination. Phytoremediation, and especially the use of trees, is an emerging and developing technology. Although its use has grown quickly in recent years, especially in the United States, Van der Lelie et al. (2001) point out the need for more well-designed and well-documented demonstration projects to promote it as a remediation technique. Local authorities, private companies and other bodies involved with the remediation of contaminated land should be encouraged to use phytoremediation, especially if budgets are limited and the alternative is that no treatment is carried out. There is an opportunity to use these sites as demonstration and research areas. Collaboration with universities, research institutes and government bodies could create the multidisciplinary teams necessary to address questions such as: the agronomic practices needed for successful establishment of vegetation, development of plants for specific remediation requirements, the question of what constitutes ‘clean-up’ (bioavailable vs. total), effects of growing plants on the wider environment and fate and disposal of high metal biomass. There is still much fundamental and applied research needed to underpin phytoremediation technology, but this could be undertaken in conjunction with actual remediation schemes, which would achieve the dual purpose of treating contaminated sites and providing demonstration sites to show the application of phytoremediation.

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