Section IV

Bioremediation

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20 Phytoremediation Technologies Using Trees

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20.1 INTRODUCTION

Regeneration of industrially contaminated land (brownfield sites) is now a priority in many countries due to risk to human health and the requirement for development land. In the U.K., for example, the government has set a target of 60% of new housing to be built on brownfield sites [1,2]. This high level of activity, however, can incur excessive cleanup costs. Traditional methods for dealing with heavy metal-contaminated soil have relied heavily upon engineering-based techniques such as:

- Excavation and removal to landfill (dig and dump)
- Encapsulation or containment *in situ*
- Separation methods, such as soil washing
- Chemical stabilization

These technologies tend to be expensive [3] and may be prohibitive, with estimates of the amount of contaminated land needing treatment increasing (estimates of up to 100,000 sites covering

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300,000 ha in the U.K.). These methods also lack environmental sensitivity. Regeneration of urban brownfield land requires innovative, low-cost, ecologically sensitive and effective techniques.

Current problems associated with reclamation of brownfield land fall into two categories: (1) disposal of existing contaminated soil cover; and (2) import of new topsoil. It is often more cost effective to work *in situ* with existing contaminated soil and to attempt to restore a healthy soil rather than to import expensive topsoil. For these reasons, natural attenuation is often favored for low-value brownfield land — sometimes combined with use of appropriate additives or amendments [4,5]. This chapter evaluates the current status of knowledge as to whether phytoremediation through tree planting may also be suited to the task [6,7].

20.1.1 The Potential Benefits of Trees

Phytoremediation, the use of plants to remove contaminants (phytoextraction) or to stabilize the soil (phytostabilization), offers a low-cost alternative that retains the integrity of the soil and is visually unobtrusive. Trees have been suggested as appropriate plants for phytoremediation of heavy metal-contaminated land because they provide a number of beneficial attributes:

- Large biomass
  - The idea that plants could take up large amounts of heavy metals into their aerial tissues and thereby clean up contaminated soil originated from the discovery and study of hyperaccumulator plants [8,9]. Such plants take up and tolerate very high concentrations of heavy metals. Their main disadvantage is their low productivity because total offtake is the product of tissue concentration and biomass yield; thus, low biomass limits their effectiveness as metal extractors. Trees, on the other hand, include many high yielding biomass species that would need to accumulate only moderate amounts of metal to be effective. Harvested trees also have more ready afteruses.
- Genetic variability
  - Because species of many fast growing, short-rotation trees, such as *Salix* and *Populus*, are genetically diverse, the opportunity exists to select genotypes with traits for resistance to high metal concentrations, or for high or low metal uptake. In Europe, there are already extensive breeding programs for *Salix*, particularly to select traits of high biomass and disease resistance [10,11]. It is evident from field observation that certain trees have no difficulty growing on contaminated sites [7]. This may be due to acclimation [12], avoidance of contaminated soil by roots [13], or accumulation in the root and limited translocation into aerial tissues [14].
- Established management practices
  - Agronomic practices for the cultivation and harvest of woody plants for biomass crops are well established, but this is not the case for hyperaccumulator plants. *Salix* (willows and osiers) and *Populus* (poplars or cottonwoods) are commonly grown in a short-rotation coppice (SRC) system, with harvesting on a 3- to 5-year cycle over a coppice life cycle of up to 30 years. Well-established forestry practices of planting, management, and harvesting have been developed with coppice on arable land, and they can readily be adapted for use on contaminated land [15–17]. Trees are typically grown at a density of 10,000 to 20,000 per hectare, producing dry matter yields of 15 odt ha^{-1} $annum^{-1}$ or higher [18].
- Economic value
  - Development of SRC systems for *Salix* has been driven by the various end uses of the trees. This has predominantly been as a biomass fuel by burning of the chipped stems, but other uses are for the production of chipboard, paper, and charcoal, and for specialist uses such as basket weaving. Progress is also being made towards more

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efficient conversion of biomass to energy fuels using anaerobic digestion, fermentation, thermochemical conversion, and improved combustion techniques [19].

- Public acceptability
  - Growing trees on contaminated land is a use that has a high degree of public acceptability and fits well into urban or rural landscapes. Urban regeneration in the U.K. and elsewhere is transforming postindustrial and derelict landscapes using multipurpose forestry to increase lowland forest cover in urban and urban fringe areas. Economic and political changes are driving the remediation of brownfield land and are starting to address the legacy of contamination from an earlier industrial history. "Low-cost" restoration to "soft end-uses" is becoming increasingly prioritized.
- Site stability
  - Trees act as a canopy protecting the soil surface from dispersal by wind or erosion by water. The roots help to stabilize the substrate. Uptake of water, and transpiration through the leaves, may help to limit the downward migration of heavy metals by leaching and thus help to protect ground- and surface waters. Leaf fall contributes a significant amount of organic material to the soil surface.

Use of trees on heavy metal-contaminated sites also has possible disadvantages. Metals may be transferred into more mobile and bioavailable forms by deposition on the soil surface following leaf fall or deeper in the soil due to root or microbial action. In general, trees tend to acidify soil, which again would cause increased bioavailability of metals. As a result of these processes, metal uptake by plants and transmission up the food chain are risks, as well as metals leaching to ground- or surface waters.

The potential use of trees in phytoremediation builds on a considerable knowledge base of metal tolerance in plants [20,21], impacts of metal contamination on trees and woodlands [22,23], and experimental studies on the responses of trees to metal contamination [24–30].

20.2 FACTORS AFFECTING UPTAKE OF HEAVY METALS BY TREES

Phytoextraction is the subset of phytoremediation that aims to maximize the uptake of heavy metals from soil into the plant. Thus, measurements of metal concentrations in plant tissue have been commonly carried out in order to assess the effectiveness of various tree species. Much, but not all, of this work has focused on the growth of *Salix* spp. in SRC [18,31–34]. *Populus*, the other genus within the same family as willows (the Salicaceae), has also been studied [35–39]. Pine [40,41], sycamore [24,26,42], birch [43–48], and oak [30] have also attracted interest. In Mediterranean climates, *Acacia retinoides* and *Eucalyptus torquata* growing on Cu mine wastes have been found to take up Cu and Pb from soil [48]. Species of *Acacia* and *Leucaena leucocephala* are potential candidates for tropical soils [49,50].

Concentrations of metals in tree tissues are affected by a number of factors; this frequently makes comparison between studies difficult. This is caused by at least five common but inherent variables, leading to errors and deficiencies that are becoming increasingly apparent.

20.2.1 SUBSTRATE VARIABILITY

Field and pot experiments have been carried out on a wide range of contaminated substrates (Table 20.1). Variations in pH, redox potential, particle size, and organic matter content, as well as the forms and concentrations of the heavy metals, mean that bioavailability of metals will be considerably different in the various substrates.

There are major deficiencies in the reliability of estimates generally used to quantify the contamination of land. These deficiencies are caused by insufficient consideration of heterogeneity of contamination within a given sampling location and thus uncertainties in the measurements. Soil

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TABLE 20.1
Various Substrates in Reported Studies of Heavy Metal Uptake by Trees

Plant growth substrate	Ref.
Soil marginally elevated in Cd	34, 90, 134–137
Sewage sludge-treated sites	31, 33, 39, 41, 42, 56, 60, 138, 139
Airborne industrial pollution	33, 83, 89, 140
Industrial/mining wastes	24, 43, 45, 58, 59, 141, 142
Landfill sites	35–38, 143, 144
Artificial contamination	46, 59

sampling is the main source of uncertainty in measurements of contamination [51] and bias is created by spatial variation of metal concentrations across the plot [52]. Standard sampling protocols potentially provide very unreliable estimates of contamination, due to problems with precision (random error) and bias (systematic error) [53]. Using bulked or composite samples clearly masks the heterogeneity of contaminant concentrations across the site, but using a number of separate sampling points for analysis of variability also has major problems. Spatial mapping of contamination and appropriate use of statistics in the design of sampling are essential before it is possible to assess whether planting trees alters metal concentrations in soil.

Using pot experiments in which soils are thoroughly mixed prior to planting is one way to evaluate the effects of tree roots on bioavailability of metals in soil, but extrapolation to the field may not be valid. In pots, the roots are forced to exploit the whole volume of soil, but in the field, plant roots may avoid hotspots of metals and proliferate in less contaminated zones [24]. In nonwoody plants, this can double the bioavailability and the bioconcentration factor (BCF) [54].

Different metals and their bioavailability also raise questions of risk. For example, 700 mg kg^{-1} of antimony (Sb) in surface soils associated with mining of this metal was found to be immobile and biologically unavailable over a wide pH range; thus, much lower concentrations of co-contaminants (As and Cu) pose a more significant risk [55].

20.2.2 Temporal Variations in Tissue Concentrations of Metals

In short-rotation coppice systems using *Salix*, a decline in tissue metal concentrations has been noted after the first year of growth [31,33]. Morin [56] found a similar decline over 3 years in a number of different tree species (ash, birch, cottonwood, maple, and pine) growing on sludged mine spoil. Such decreases in metal concentrations may be partly attributed to dilution due to growth, but may also be due to relatively rapid rates of uptake from soil during the first year, when trees are growing quickly and roots are becoming established.

In addition to this year-to-year variability, many workers have measured variations in the metal contents of various tissues in trees within a single growing season [18,32,44,57–60]. On highly contaminated sites, an increase in metal concentrations in leaves has been interpreted as a survival trait whereby toxic metals can be lost from the tree at leaf fall. Redistribution of metals from leaves and bark to wood at the end of the growing season has also been recorded in trees growing on moderately contaminated sites [32].

20.2.3 Variability between Component Parts of Trees

Many studies have noted variation in metal concentrations between different tissues and organs of trees [14,18,24,31,39,42,56,59–64]. In general, the order of metal concentrations is roots > leaves > bark > wood, although different elements show different patterns of distribution. Lead, Hg, and

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Cr, especially, are immobilized in the roots, whereas Cd, Cu, Zn, Co, Mo, B, and Ni tend to be found more distributed to the other tissues, reflecting their greater mobility within the tree.

Knowledge relating to uptake patterns of heavy metals in trees is still very limited, but it is known that metals tend to remain located in the roots rather than being transported to aerial tissues [43,65]. Heavy metals in soils may or may not enter the root tissues and then move to the xylem [66,67]. Onward mobility and translocation through the plant also differ between metals and between plants. Accurate modeling of uptake and fate of trace elements in plants has not yet been achieved, but clearly depends on solubility and mobility.

Uptake (and toxicity) depends on ability of trace elements to cross membranes and to be transported. Ionic status and organic complexation are particularly important. For example, the inner lumen walls of xylem elements are lined with fixed negative charges that bind cations, removing them from the transpiration stream as they move up the xylem. Monovalent ions (e.g., K^+) move at the same rate as the transpiration stream, but divalent ions (e.g., Ca^{2+}) move slowly. Divalent ions bind preferentially to the fixed negative charges, displacing the monovalent cations (which move back into solution). However, some metals (e.g., Cu^{2+}, Ni^{2+}, and Fe^{3+}) move at the same rate as the transpiration stream because it contains dissolved organic molecules (amino acids, organic acids, sugars). Changes occur in speciation as they form complexes with organic molecules (and may become negatively charged); for example, Cu and Ni bind with amino acids.

20.2.4. Toxicity of Trace Elements to Plants

Background concentrations of metals available for uptake in soil are variable, as are the plant tissue concentrations associated with toxicity. Experimental evidence for minimum toxic concentrations of heavy metals known to affect trees was reviewed by Pahlsson [68]; unfortunately, most of it relates to short-term studies of seedlings grown in artificial culture. The results vary greatly according to whether metal salts were supplied in a growth medium of solution, sand, or soil, and between species and soil types. For example, in one study of boron toxicity, eight tree species were grown in sand receiving nutrient solution containing elevated boron concentrations from 0.2 to 20 mg l^{-1}; however, despite some foliar symptoms of toxicity at 5 mg l^{-1} and above, growth was not severely retarded even at 20 mg l^{-1} [69]. Otherwise, no comprehensive study of boron toxicity in woody species exists in the literature; neither soil nor plant analysis can be used to predict precisely the growth of plants on high B soils [70].

Mobility varies between species and in relation to different metals, but some generalizations can be made. There are two basic groups in relation to mobility in plants: (1) trace elements immobilized in roots, e.g., Al, Cr, Hg, Pb, Sn, and V; and (2) mobile trace elements, e.g., As, B, Cd, Cu, Mn, Mo, Ni, Se, and Zn. The mobile elements may be phytotoxic, giving reduced growth (As, B, Cu Mn, Ni, and Zn) or may accumulate with no discernible effect on plants (Cd, Mo, Se, Be, and Co). This suggests that the most scope is for targeting the latter trace elements for phytoextraction.

20.2.5 Plasticity of Plant Response to Metals

In the field, it is increasingly apparent that trees can survive and grow in contaminated soil with exceptionally high levels of multiple metals [22,23,31,71–73]. Seedlings may be more sensitive than saplings or mature trees, and metal concentrations in seedlings are often higher than in mature plants [74]. Tree roots can actively forage towards less contaminated zones of soil [25] and, even with highly reduced growth, they can "sit and wait" for favorable growth conditions [75]. Trees are able to grow and survive by being resilient and, undoubtedly, they have a large capacity for phenotypic adjustment to metal stress [4,27,29,76].

Metal tolerance to zinc and lead has been identified in populations of birch and willow [5,77,78]. Eltrop et al. [45] found *Salix caprea* (goat willow) growing in soil with total concentrations of

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17,000 mg Pb kg^{-1} (with NH_4Oac extractable concentrations of 4000 mg Pb kg^{-1}) and *Betula pendula* (silver birch) in soil with total concentrations of 29,000 mg Pb kg^{-1} (extractable concentrations of 7000 mg Pb kg^{-1}). Established woodlands have been shown to remain healthy following industrial fallout, which has raised total soil concentrations above 10,000 mg Cu kg^{-1} and 100 mg Cd kg^{-1} [22,79].

Despite this ability to survive contamination, results from other experimental studies have been extrapolated and it has been concluded that, in some soils, heavy metals may sufficiently inhibit growth of trees to be of significance to commercial forestry [80]. The best direct evidence is from situations in which trees have been planted on reclaimed metalliferous mining spoils [43]. In many soils, it is likely that elevated concentrations of available metals could be ameliorated prior to planting to avoid such effects; for example, liming and fertilization have been found to affect the heavy metal concentrations in *Pinus sylvestris* (Scots pine) [81]. Substantial variation exists, even within clones of the same species [12,82].

20.3 FACTORS AFFECTING METAL OFFTAKE DURING HARVEST

Further recent advances in knowledge also help to evaluate the likelihood of using trees for phytoremediation.

20.3.1 Recycling Metals to the Surface of Soil

Tree foliage can contain high concentrations of heavy metals, especially Cd and Zn. On highly contaminated sites, these may be at their peak values just before leaf senescence, appearing not to be translocated and redistributed within the plant prior to leaf fall. If these metals return to the rhizosphere, they represent a significant pool of potentially bioavailable metal. Under such circumstances, phytoremediation will fail in its aim of cleaning up the soil. It does, however, raise the question of improving offtake of metal by harvesting the trees prior to leaf fall. This is not normal practice in SRC systems in which the stems without leaves are harvested over the winter period. With long-term tree covers that are not regularly harvested (and in the years intervening in the usual 3-year SRC harvest), this recycling process of metals to surface soil may be significant.

20.3.2 Metals Located in Tree Roots

Roots usually contain the highest concentrations of all metals in trees; depending on the size of the root biomass, this could be a significant amount. Death and decomposition of roots during normal growth processes or following harvest of above-ground biomass probably release heavy metals back into the rhizosphere. Because the roots can contain a significant pool of metals, it may be that the root bole should be harvested at the end of the SRC cycle (after 25 to 30 years) or when a mature tree is cut down.

Root density and the depth of rooting are particularly significant in the context of phytoremediation [83]. Studies on 246 coppice stools of five *Salix* and five *Populus* clones in four different soil types at seven sites in the U.K., with a stool age of 3 to 9 years, found rooting to a depth of more than 1.3 m; however, 75 to 95% of roots were in the top 36 cm. Wetter soils had shallower root systems [84]. It has also been found that *Salix cinerea* has a much reduced uptake of Cd and Zn into leaves and bark when grown in wetland compared to that in drier soils [85].

20.3.3 Metals Located in Stems

Although the two are often reported together simply as “stem” tissue, tree bark usually contains a higher concentration of metals than wood does [14,86–88]. The higher biomass of wood means that it contains a greater pool of metal than the bark does. Wood and bark (stem) tissue contains

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a significant pool of heavy metals (concentration × biomass yield), one that increases with the age of the tree. This pool of metals is much less bioavailable than those in the roots and leaves. Regular cutting of trees in the SRC system is designed to increase stem biomass, but also increases the ratio of bark to wood and thus may increase this pool of immobilized metal.

20.3.4 Modeling Metal Offtake

Uptake data are frequently converted to BCFs that allows a better comparison to be made of the metal uptake abilities of trees grown under different conditions.

$$\mathrm{BCF} = \frac{\text{concentration in plant tissue (mg kg}^{-1}\text{)}}{\text{concentration in soil (mg kg}^{-1}\text{)}}$$

Care must be taken when collating such data because of the different strengths and types of acids and extraction processes that are routinely used to measure soil concentrations of heavy metals (see Section 20.4.1). Table 20.2 shows recently published BCFs for uptake of Cd, Cu, Ni, Pb, and Zn by trees. BCF values > 1 indicate that metal is accumulated in the tree relative to the soil.

This approach suggests potential for enhanced uptake of Cd by *Salix*, especially when only slightly elevated concentration of Cd is in the substrate. Zinc uptake may also be significant but, in view of limited zootoxicity of this metal, is of less concern on brownfield land. In general, transfer of Cu, Ni, and Pb from soil to plant is very poor.

Although BCF values can give a guide to the uptake of metals from soil, the concentration of a metal in plant tissue does not alone give any information about the absolute amount of metal removed from the soil. Knowledge of the biomass yield is also required in order to calculate metal offtake (concentration × yield). Some estimates of metal offtake obtained from field trials using *Salix* are given in Table 20.3. Some of the trials measured offtake over a number of years; these have been corrected to an annual figure to allow comparison between trials of differing duration. As pointed out earlier, yearly values may not be equal and, with coppiced systems especially, the effects of tree growth and harvest may be important.

Such figures do, however, show some consistent features. Cadmium, Cu, Ni, and Pb are removed at the rate of the order of tens of grams per hectare per year, whereas the value for Zn is about 100 times higher. When compared with estimates of the metal content of the soil to rooting depth, such offtake figures generally give estimates of hundreds to tens of thousands of years to reduce the soil metal contents to acceptable values. Only the case of Cd, in which the amount taken out by the trees can be a significant percentage of the soil metal, offers hope of cleanup within a realistic time scale (Table 20.4).

20.4 OUTSTANDING ISSUES

20.4.1 Importance of Bioavailability of Heavy Metals in Soil

Soil contamination guidelines tend to be based on some measure of acid extractable metal. Measurement of a true total metal content in soil requires dissolution of the whole of the solid soil matrix, which, because the predominant mineral type in most soils is silicate, involves the use of hydrofluoric acid (HF). For safety reasons, there is a tendency not to use this acid and thus a "pseudototal" value is often obtained using less aggressive reagents. This means that the "total" values reported in the literature have been obtained by various methods of differing extracting power (Table 20.5). Therefore, care must be taken when these data are interpreted.

One measure of the success of phytoremediation is the decrease in soil metal content resulting from tree growth. It could be argued that total (or pseudototal) metal values are meaningless in

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TABLE 20.2
Bioconcentration Factors Reported in the Literature for Trees Growing on Contaminated Land

	Cd	Cu	Ni	Pb	Zn	Ref.
Alnus incana						**143**
Leaves	—	0.05	—	—	0.07	
Twigs	—	0.03	—	—	0.06	
Betula pendula						**143**
Leaves	0.06	0.02	—	—	0.37	
Twigs	0.11	0.03	—	—	0.32	
Fraxinus excelsior						**143**
Leaves	—	0.01	—	—	0.02	
Twigs	—	0.04	—	—	0.05	
Salix viminalis						**143**
Leaves	0.83	0.01	—	—	0.37	
Twigs	0.72	0.03	—	—	0.28	
Sorbus mongeotii						**143**
Leaves	—	0.01	—	—	0.03	
Twigs	—	0.02	—	—	0.05	
S. viminalis "Orm"						**145**
Roots	1.1	0.2	—	0.1	0.5	
Wood	1.2	0.1	—	0.1	0.3	
Leaves	1.4	0.1	—	0.02	0.8	
S. viminalis clone 78183						**134**
Stem	2.9–16.8	—	—	—	—	
Leaves	4.8–27.9	—	—	—	—	
***S. viminalis* clone 78198**						**33**
Calcereous soil — stem	0.65–1.57	—	—	—	0.11–0.31	
Calcereous soil — leaves	1.61–2.74	—	—	—	0.59–1.71	
Acid soil — stem	0.79–0.93	—	—	—	0.06–0.08	
Acid soil — leaves	1.29–1.82	—	—	—	0.23–0.36	
20 *Salix* clones						**31**
Stem	0.0–0.45	0.01–0.03	0.01–0.06	—	0.04–0.15	

this context and that some measure of change in the bioavailability of the metal should be used. There have been two broad approaches to this. First, single selective extractants, which have been used extensively in the study of soil chemistry, are employed to give an assessment of bioavailability. This approach assumes the model of a large pool of non-, or partly, bioavailable metal and a smaller pool of bioavailable metal, upon which the plant roots draw. Because this more available metal is removed by plant uptake (or by leaching), it may be slowly replenished by weathering of the larger, nonavailable pool (Figure 20.1).

Because the bioavailable pool contains the environmentally active metal, it could be argued that phytoremediation is successful if it depletes this pool. This argument has two uncertainties: the rate at which metal moves from the nonavailable to the available pool, and the return of metal

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TABLE 20.3
Offtake Estimates by *Salix*[a]

	Cd	Cu	Ni	Pb	Zn	Ref.
Dredged river sediment	12	38	—	57	795	145
Coppice soils (estimates based on 25 years)	2.6–16.5	—	—	—	—	134
Calcareous soil pH 7.3 (average over 5 years)	34		—	—	2680	33
Acid soil pH 5.2 (average over 2 years)	24		—	—	7250	33
Calcareous soil, 7.3 (3-year-old trees)	44	187	—	—	3851	83
Sewage sludge soil, pH 6.3 (maximum value after 1 year's growth)	62	59	37	—	822	31
Assuming biomass production of 15 odt/ha/yr — stems	33	—	—	—	—	136
If leaves are also harvested	61.6	—	—	—	—	136
Assuming 10 odt/ha/yr	34.3–76.7	58.6–81.5	9.3–13.8	—	954–1560	18

[a] Grams of metal per hectare per year.

TABLE 20.4
Number of Years to Reduce Soil Cd Concentrations by 5 mg kg^{-1} by Planting *Salix* to Different Soil Depths[a]

	Plant tissue concentration (mg kg^{-1})			
Depth (cm)	**10**	**25**	**50**	**100**
10	33	13	7	3
20	67	27	14	7
40	133	53	28	14

Note: Calculated according to rates of metal uptake into above-ground plant tissues. Calculation assumes (1) yields of 15 ODT ha^{-1}; (2) bulk density of 1; and (3) consistent Cd uptake throughout the period. Fertilization and irrigation may be required to achieve these yields.

TABLE 20.5
Extracting Procedures Used to Measure Total or Pseudototal Metal Concentrations in Contaminated Soil

Acid extraction scheme	Ref.
2M HNO3	33, 83, 90, 143
7M HNO3	34, 134–136
HCl + HNO3 (3:1)	31, 45, 146
HNO3 + HCl (3:1)	147
HNO3 + HCl + HClO4 (3:3:1)	46
HNO3 + HF + HClO4	89
HNO3 + HCl + HF microwave digestion	145

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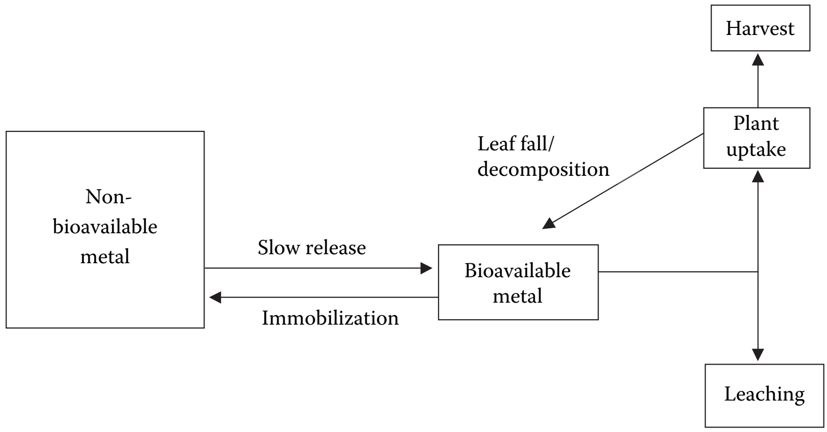

FIGURE 20.1 Model of bioavailability of metals in soil.

to the soil by leaf fall or plant decomposition. Measures of the bioavailable pool have been made by three broad approaches:

- Measurement of metal in soil solution, which is the source of all metal taken up by plant roots
- Extraction by simple salt solutions, e.g., 0.01 *M* $CaCl_2$, 0.1 *M* $NaNO_3$, 1 *M* NH_4NO_3
- Extraction by a metal complexing reagent, e.g., 0.05 *M* EDTA, 0.005 *M* DTPA

The second way in which the changes in soil metal chemistry are studied is by use of sequential extraction. This assumes that metals are held in soil in a series of pools of decreasing bioavailability and uses increasingly aggressive reagents to remove successive pools of metal. This approach allows study of changes in the distribution of metal held in different ways.

Published results of bioavailability of metals measured by single extractants have shown mixed results. Hammer and Keller [89] used three separate extractants (0.1 *M* $NaNO_3$; 0.005 *M* DTPA/0.1 *M* TEA in 0.01 *M* $CaCl_2$, pH 7.3; 0.02 *M* EDTA/0.5 *M* NH_4OAc, pH 4.65) to assess the bioavailability of Cd, Cu, and Zn in two contaminated Swiss soils; one was calcareous (pH 7.78) and one was acidic (pH 5.27). Under *Salix viminalis,* they measured a significant decrease in $NaNO_3$ extractable Cd and Zn in both soils, but DTPA–Cd only in the acidic soil, compared with the unplanted soil. Conversely, they found an increase in DTPA–Zn and Cu in the acid soil.

Eriksson and Ledin [90] used 1 *M* NH_4NO_3 and 0.01 *M* $CaCl_2$ to measure the bioavailability of Cd under *Salix viminalis* in Swedish soils and found a decrease in the planted soils. Pulford et al. [31] used 0.05 *M* NH_4EDTA to extract an organic (LOI 22%; pH 6.3) sewage sludge-treated soil following coppicing of the trees. In plots where the most productive clone (*Salix aurita* x *cinerea* x *viminalis,* Rosewarne White) had grown, extractable Cd, Cu, Ni, and Zn were higher than in adjacent unplanted areas.

Watson [32] used 1 *M* NH_4OAc (pH 6) and 0.025 *M* NH_4EDTA (pH 4.6) to measure bioavailability of metals under five *Salix* clones growing on a sewage disposal site (LOI 9.9%, pH 6.2) compared to unplanted areas. No significant effect on the NH_4OAc extractable metals was found, but the EDTA extractable metals were lower under the trees. On another sewage sludge site (pH 5.4 to 5.8), Watson [32] measured the following increases in the concentrations of metals in the soil solution on planted sites compared to unplanted: Cd: +133%; Cr: +9%; Cu: +50%; Ni: +51%; Pb: +58%; and Zn: +102%.

The second approach, sequential extraction, is useful for allocating metal fractions in soil, but the process is operationally defined, so interpreting these in terms of bonding to specific soil components should be avoided. A large number of extraction schemes of varying complexity have

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been used for natural soils and some have been applied to contaminated soils to assess the effects of tree growth on metals. Hammer and Keller [89] used a six-step extraction scheme based on that of Benitez and Dubois [91]:

1. 0.1 *M* $NaNO_3$
2. 1 *M* NaOAc, pH 5
3. 0.1 *M* $Na_4P_2O_7$
4. 0.25 *M* $NH_2OH.HCl$ + 0.05 *M* HCl
5. 1 *M* $NH_2OH.HCl$ + 25% HAc
6. HF, HNO_3, $HClO_4$

They found that Cd was extracted predominantly in step 2 for the calcareous soil and step 3 for the acid soil; Cu was predominantly removed in step 3 for both soils. Zinc was more evenly distributed over a number of steps: 2, 3, 4, and 5 for the calcareous soil; and 3 and 5 for the acid soil. Only very small changes in the distribution of metals among the six steps were found after growth of *Salix*. Slight, but significant, decreases were measured for Cd step 3 and Cu step 4 in the acid soil; Zn step 4 in the calcareous soil; and steps 1, 3, and 4 in the acid soil.

Watson [32] used the four-stage BCR sequential extraction scheme of Davidson et al. [92] on a calcareous sewage sludge disposal site (pH 7.1 to 7.8):

1. 0.11 *M* HAc
2. 0.1 *M* $NH_2OH.HCl$, pH 2
3. 8.8 *M* H_2O_2/1 *M* NH_4OAc
4. Aqua regia

Cadmium and zinc were extracted mainly in steps 1 and 3; Cu and Pb mainly in step 3; and Ni and Cr in steps 3 and 4. Step 2 extracted only very small amounts of all six metals.

In the case of single and sequential extraction, care must be taken in the interpretation of any changes in metal extractability. For example, the increases in EDTA-extractable Cd, Cu, Ni, and Zn reported by Pulford et al. [31] were measured in soil after coppicing of willow. Cutting of the trees could trigger changes in the rhizosphere (for example, due to root death) that could cause the release of low molecular weight organic compounds, which could complex the metals resulting in greater extractability. Changes measured under growing trees tend to show a decrease in EDTA-extractable metals, which may be due to plant uptake.

Overall, the few studies of this type reported do tend to suggest that tree growth causes changes in the bioavailable pool of metal, shown particularly by the increase in soil solution metal concentrations measured by Watson [32]. Whether an increase or a decrease in bioavailability is measured depends on the stage in the growth cycle when the measurements are made, i.e., the balance between plant uptake and retention of metal in the rhizosphere and the multiple additional factors described earlier.

20.4.2 Root Foraging and the Rhizosphere

Soil heterogeneity has a large influence on the location of root activity and growth in soil. Plant roots selectively forage favorable regions in the soil and, similarly, may avoid unfavorable or toxic zones [24]; this has been found to have a profound effect on metal uptake by plants [54]. In low-level or moderately contaminated soils, of course, some elements (e.g., Cd) may be undetectable by plant roots. Furthermore, root processes may alter the speciation and mobility of metals. Phytoavailability of metals is largely related to the aqueous speciation and free ion activity of that metal. The free metal ion is in rapid equilibrium with cell-surface binding sites, and is thus available

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for uptake; however, other metal species (e.g., colloidal metals and metals complexed to strongly binding organic ligands) are not available for plant uptake (Figure 20.1) [93].

Clearly, there is much scope for transformation of metals between labile and nonlabile pools and for changes between aqueous- and solid-phase metals. The role of rhizosphere processes in metal mobility and availability is poorly understood, but root exudates are likely to have a major influence on speciation and metal bioavailability. For example, investigations of Ni hyperaccumulator plants in rhizotrons have shown that exudation of organic ligands from roots forms Ni–organic complexes that enhance the solubility of the metal in the rhizosphere and increase Ni uptake by the plant [94].

Mycorrhizae are associated with the roots of most of the higher plants, and long-lived woody species are almost invariably mycorrhizal [95]. Over 150 taxa of mycorrhizae have been found to be associated with *Salix* alone in Britain [96]. Mycorrhizal fungi are known to be influential in affecting establishment of trees on nutrient-deficient and severely contaminated soils [97] and in limiting uptake of metals by tree roots from soil. It has been argued that mycorrhizae provide an effective way for tree roots to survive in metal-contaminated soils [98]. Much shorter life cycles offer much more opportunity for selection of metal resistance in mycorrhizae, avoiding the necessity for an adaptive response of tree roots and allowing their survival in otherwise toxic soils.

There is little doubt of the protective role of mycorrhizae in metal-contaminated and otherwise environmentally stressed soils; they provide benefits of enhanced nutrient uptake while offering protection against uptake of toxic metals. However, the role of mycorrhizae is less certain in soils that contain elevated concentrations of metals that are not necessarily toxic or detrimental to growth of the plant or fungus. Ericoid mycorrhizae are known to improve the ability of plants to grow on metal-contaminated soils. In unpolluted soils, the fungus increases the solubility and mobility of Zn using extracellular organic acids. However, in Zn-polluted soil, the fungus loses this ability and protects the plant from excessive Zn uptake [99]. Thus, the fungus appears to maintain homeostasis of metals under different soil conditions. Uptake of Cd, Ni, and Pb from contaminated soils has been shown to be lower in *Quercus rubra* (red oak) seedlings infected with the ectomycorrhiza *Suillus luteus* [100].

Soil processes that influence pollutants can be complex. One example is from evidence that *Pinus sylvestris* (Scots pine) roots and associated mycorrhizal infections increase soil bacterial communities that, in turn, enhance the degradation of mineral oils and petroleum hydrocarbons (PHC) in contaminated soil [101]. Whether mycorrhizae increase or decrease uptake of metals by plants is uncertain, but this is of obvious relevance to remediation of contaminated soils [102]. Ectomycorrhizae have been shown to increase and decrease uptake of Cd by Norway spruce (*Picea abies*) seedlings, depending on the concentration of the metal to which the seedling is exposed [103]. Practical application of this knowledge by inoculating trees roots is only in the rudimentary stages, although mycorrhizae may be less abundant and of less importance on fertile soils [98,103–107].

20.4.3 Other Ecological Processes in Soil

The role of soil fauna, such as earthworms, in altering the mobility of metals in soils in relation to tree growth is equally little understood, although their presence in soil can markedly increase tree growth [108]. Earthworms are intricately involved with the activity of living roots through decomposition, soil microbial activity, and mineralization [109]. It has long been suspected that earthworms are likely to alter the solubility of heavy metals in soil, but this is largely unexplored in the literature.

Ireland [110] found that *Dendrobaena rubida* altered the solubility of Pb, Zn, and Ca in reclaimed Welsh mining spoils, reporting a 50% increase in availability of water-soluble Pb in feces. Devliegher and Verstraete [111,112] found that availability of copper, chromium, and cobalt

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was raised by between 6 and 30% in the soil by *Lumbricus terrestris*. Ma et al. [113,114] found that species of *Pheretima* increased the mobility of metals in Pb/Zn mine tailing diluted with uncontaminated soil. They suggested that earthworms may benefit attempts to use plants for phytoextraction by increasing the amount of metal in the soil available for plant uptake. The presence of *L. terrestris* in microcosms increased the concentration of Cd, Cu, and Zn into roots and shoots (and of Pb and Fe into roots) of *Lolium perenne* seedlings [115,116].

Experimental work has shown that earthworm activity enhances tree seedling growth associated with enhanced soil organic matter, improved nutrition (including NO_{3-}, NH_{4+}, and Ca^{2+}), and increased mycorrhizal colonization [117]. Yield of a tropical leguminous woody shrub, *Leucaena leucocephala*, in amended Pb–Zn mine tailings has been found to be increased by 10 to 30% in the presence of burrowing earthworms (*Pheretima* spp.) [113,114]. The earthworms increased available forms of N and P in soil, increased metal bioavailability, and raised metal uptake into plants by 16 to 53%.

Some evidence indicates that earthworms increase metal bioavailability in relatively low-level metal-contaminated soils with higher organic matter contents [114,118]. This agrees with results of experiments in which the addition of exogenous humic acid to soil has been shown to increase plant-available metals [119]. This appears to be due to the formation of metal–humic complexes that prevent transformation to insoluble species. If this is correct, clearly earthworms may be of considerable benefit to phytoextraction. The current state of knowledge certainly suggests that phytoremediation should be viewed in an ecological context that includes a consideration of the plant–soil–animal interactions that influence metal mobility.

20.5 PHYTOEXTRACTION VS. PHYTOSTABILIZATION

From the evidence of the BCFs listed in Table 20.2, only Cd and Zn will accumulate in sufficiently high concentrations in the above-ground tissues of trees (BCF > 1) to be candidates for phytoremediation. When the amounts accumulated are compared with the amounts of metal in the soil, only removal of Cd from marginally contaminated soil is possible within a realistic timescale. Zinc is usually present in high concentrations, of which the amount of metal in the plant tissues represents a very small proportion. The other metals commonly studied (Cr, Cu, Ni, Pb) are poorly bioavailable in the soil or are poorly translocated out of the root.

Willows accumulate Zn and Cd more readily than other trees and woody plants do and it has been suggested that this may pose a threat of transfer of metals to the food web [120,121]. It has also been demonstrated that Zn can be transferred to aphids via plant uptake, resulting in Zn concentrations in aphids four times greater than in the soil [122]. Cadmium concentrations in aphids reflected those in plants, but neither metal appeared to be transferred to predatory ladybirds. High Cd concentrations in tissues of small mammals have been recorded at similar sites where willow is grown on dredged sediments [123].

Alternatives to phytoremediation include *ex situ* washing methods with strong chelating agents such as EDTA [124] or acids [125]. Attempts to use the same chemicals *in situ* to enhance metal uptake into plants have had limited success [126], are often prohibitively expensive, and may be destructive to fertility and soil biota; also, residual concentrations in soil pore water may pose a risk of ground water contamination. Nevertheless, BCFs can vary by an order of magnitude over a relatively short pH range from 5.5 to 7.0 in leaf and root vegetables [127], and Cd uptake into *Salix* has been found to be highly pH dependent in field stands [90]. It may be possible to enhance Cd uptake with low-cost organic or inorganic acid soil amendments.

Considerable potential benefits can be gained from the growth of trees on contaminated land, resulting from the stabilization of the soil and/or the contaminant. The protection afforded simply by the presence of the large above-ground biomass of trees can result in a decrease in wind- and water erosion of the soil [128]. Leaf litter can accumulate on the soil surface, forming a barrier over the contaminated soil, which can also help its physical stabilization. Tree roots form a

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significant below-ground biomass that can effectively bind the soil [129]. They also take up large amounts of water lost from the leaf surface in the transpiration stream. This represents a significant upward movement of water from the soil, via the plant, to the atmosphere, which decreases the potential for metals leaching from the soil. It was estimated in one study that leaching under a tree cover was about 16% less than under grass [130].

In addition to the physical stabilization, chemical stabilization of certain elements may occur. For example, it has been shown that Cr is strongly held in the roots, regardless of the form supplied to the plant (CrIII or CrVI). CrIII is highly insoluble and thus poorly bioavailable, and it has been suggested that CrVI can be reduced to CrIII in the rhizosphere [131]. Lead is another element that is strongly bound in root tissues, possibly due to the formation of lead phosphate [132].

In addition to the use of various soil amendments, including liming, zeolites, beringite, and organic matter, tree cover provides a potentially sustainable vegetation that may allow the contaminants to remain permanently immobilized in soil or woody biomass. At the present time, evidence demonstrating that this happens is insufficient. It is known that metals can become vertically mobile in soil profiles under mature woodlands [22,79] and, clearly, this may threaten underground aquifers in the longer term. Other studies of highly contaminated mature woodlands [133] have demonstrated very low metal mobility and relatively steady-state conditions. Further studies are required to evaluate the long-term feasibility of using trees to stabilize metal contaminants in soil.

20.6 CONCLUSIONS

Urban regeneration, remediation of brownfield land, and cleanup of contaminated soils require innovative, low-cost, ecologically sound, and effective techniques for removal of heavy metals from soil. Phytoremediation using trees provides a potential opportunity to extract or stabilize metals. Phytoextraction involves the use of high yielding plants that readily transport targeted metals from soil to vegetation, allowing removal of metals by harvesting the plants, without damaging the soil or requiring its disposal to landfill. The process takes longer, but meanwhile allows greening of the land, and harvested plants can be used as bioenergy crops.

We provide gathering evidence that selected naturally occurring clones of *Salix* (willows and osiers) are likely to meet this requirement for cadmium, resolving risks associated with its widespread and ubiquitous contamination of soils and restoring soil health within a realistic time. When the concentration of Cd in a soil is just above guideline values or annual inputs are small, phytoextraction by *Salix* may provide an efficient and cost-effective method of cleanup. However, despite many claims to the contrary, field evidence is lacking and phytoextraction using trees remains an unproven technology for reasons discussed. There is probably very limited scope for other metals on brownfield land, although opportunity to reduce phytotoxic concentrations of Zn from agricultural soils may exist.

Tree planting provides aesthetic and ecological improvement of derelict and underused land. The likelihood of the success of phytostabilization is increasing and a good range of field-based evidence already exists. However, outstanding gaps in knowledge relate to the long-term success of ensuring no risk to human health (from re-entrainment of particulates or increased downward movement of metals to water bodies) or to the wider environment (e.g., to food chains).

The probable outcome of current research efforts will be that both phytoremediation strategies using trees will form part of an integrated solution to brownfield land, alongside natural attenuation, soil washing, and chemical and biological additives to contaminated soils.

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