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Chapter

PHYTOREMEDIATION AND PHYTOMINING: USING PLANTS TO REMEDIATE CONTAMINATED OR MINERALIZED ENVIRONMENTS

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ABSTRACT

One type of harsh environment for plants is metal- and metalloid-contaminated or mineralized soils: these exist in most countries due to geological formations or to a history of mining and/or smelting. Depending on soil pH and fertility, metal-rich soils may be barren and eroding into wider areas. Some elements present risk to humans, wildlife, livestock, plants, or soil organisms and require remediation. The engineering approach of removing the contaminated soil is extremely expensive. Thus, alternative methods for *in situ* remediation of element-rich soils have been developed by the agricultural sciences. These methods include phytoextraction (growing plants which accumulate high concentrations of an element in shoots for removal from the field) and phytostabilization (adding soil amendments which convert soil elements into forms with

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much lower phytoavailability and bioavailability so they no longer pose a risk to the environment). Phytomining is a variant of phytoextraction in which the element accumulated in plant shoots has enough value to support farming a hyperaccumulator crop to produce a commercial bio-ore. This chapter reviews these valuable phytotechnologies which have been developed in the last few decades to reduce the costs of alleviating environmental risks of contaminated soils.

INTRODUCTION

Phytoremediation comprises a group of technologies which may be used to reduce risks from elements (metals and metalloids) and biodegradable organic compounds in contaminated or mineralized soils. Phytoremediation includes phytoextraction, the use of plants to remove elements from soils into shoots to decontaminate a soil. If the elements accumulated in plants have no economic value and the biomass, ash or compost of the plant materials must be handled as a hazardous waste and placed into a landfill or recycled, it is simple phytoextraction. Phytomining uses plants to recover soil elements in above-ground biomass which then has value in markets; for example, biomass is ashed and the ash marketed as a high-grade bio-ore. Rather than removing the contaminant, phytostabilization uses plants and soil amendments to convert soil contaminants to forms that are no longer sufficiently bioavailable or phytoavailable that they have adverse effects on plants, animals, or soil organisms. Soil amendments can promote the formation of more strongly adsorbed, precipitated or occluded forms of the contaminants, depending on the chemistry of the specific contaminant and amendments (Chaney et al., 2010; Scheckel et al., 2009). Plant roots can promote formation of less soluble forms of Pb (Cotter-Howells et al., 1999) and plants hold soil in place to prevent erosion, which could cause more extensive contamination. Phytoremediation may also include phytodegradation of soil xenobiotic compounds by plants or rhizosphere microbes (not covered in this review).

Perhaps the first use of soil metal phytostabilization is the work of Bradshaw (1975) and Smith & Bradshaw (1970; 1972) to use minimal soil amendments (fertilizers) to revegetate mine wastes using metal-tolerant ecotypes of native grasses. Gadgil (1969) showed that, by combining application of organic amendments with metal tolerant ecotypes, even better revegetation could be achieved. Baker (1981) reviewed this knowledge and that of Cannon (1960) and Ernst (1974) which communicated to new generations these ideas about metal tolerance and the important distinction between accumulation and exclusion (see Ernst (2006) for a recent review of the evolution of metal-tolerant plants). One difficulty of using metal tolerant grasses in remediation is the need for continuing N fertilization to maintain plant cover. Until recently there were no known legumes with appreciable metal tolerance in acidic Zn contaminated soils. *Anthyllis vulneraria* var. *carpatica* (Pant) Nyman (Fabaceae) has recently been found to tolerate Zn and fix N in Zn/Pb mine wastes in France (Mahieu et al., 2011; Soussou et al., 2013). Even if a metal-tolerant grass could be established with this Zn-tolerant legume, persistence of the plant cover would require additional fertilization (P, K) and would strongly benefit from limestone incorporation if the soil is non-calcareous. N-fixation generates soil acidity over time which increases Zn phytoavailability and could eventually cause phytotoxicity if excess limestone is not included in the phytostabilization practice. The decline in pH and reduction in plant survival and seedling establishment over time is illustrated in forest soils near the Palmerton, PA (USA) Zn smelter (Beyer et al., 2010; 2013). For highly metal contaminated soils it is often necessary to make the soil calcareous to avoid a subsequent drop in pH and loss of vegetative cover, and hence the reversal of the remediation (see Chapter 14).

Inexpensive persistent remediation will usually require the use of soil amendments to alleviate metal toxicity and any nutrient deficiencies to aid plant growth and reduce risks for wildlife and livestock feeding upon the plants. For example, some soil treatment is often needed to convert soil Pb to forms which have lower bioavailability to animals when soil is ingested (see below). For a few elements which are not easily accumulated in plant shoots in phytostabilized soils (*e.g.,* As, F, and Pb), the inadvertent ingestion of soil by wildlife or children is the dominant route for risk (Basta et al., 2005; Chaney, 1983). Chaney (1983) introduced the 'Soil-Plant Barrier' model to summarize the overall patterns of risk from soil elements. Some elements are so insoluble in soil or immobilized in plant roots (*e.g.,* Cr and Pb) that they do not reach food-chain dangerous levels in plant shoots for humans, livestock, or wildlife. Other elements (*e.g.,* As, Cu, F, Mn, and Ni) are sufficiently phytotoxic, and animals sufficiently tolerant, that even plants suffering element phytotoxicity do not cause adverse effects on the most sensitive animal species. Under some soil pH conditions, a small group of elements can be absorbed and translocated by plants from contaminated or mineralized soils to poison livestock, wildlife, or humans (Cd, Mo, and Se).

When an element in the contaminated soil has enough economic value, and plants exist which hyperaccumulate the element, it is possible to establish phytomining to alleviate the environmental risk from such sites and over time to improve the original fertility of the soils to support farming as a byproduct post phytomining. But if the value of the element in biomass is low it cannot pay for the remediation service and companies doing the phytoremedation will need to be paid. In many cases, phytostabilization will be the method of choice due to low costs compared with phytoextraction when the biomass has insufficient economic value. Consideration of the amount of metals in a contaminated soil, and the inability of normal plant species to accumulate high levels of metals without strong phytotoxicity, illustrates why the plants called 'hyperaccumulators' are necessary for functional phytoextraction. Hyperaccumulators accumulate about 100-fold higher metal concentrations than normal plants, and often 1,000-fold higher metal, on mineralized or contaminated soils (Chapter 10; van der Ent et al., 2013).

SOILS WHICH REQUIRE REMEDIATION

Acidic Cu, Ni, Pb, and Zn mine waste and smelter-contaminated soils are often severely phytotoxic and require remediation or revitalization. At many of these locations ecosystems have been destroyed and barren soils are eroded, so rather than 'ecosystem restoration,' we consider 'revitalization' of such sites. In some locations, mine wastes contain pyrite which generates strong soil acidity during its oxidation, causing dissolution of soil Mn and Al minerals to reach phytotoxic levels for crop plants (pH<5.2): soil pH may become as low as <3.0 so that combined metal toxicity inhibits all but the most metal-tolerant plant species/ecotypes. Extensive areas of Zn-Pb mine or smelter waste or smelter contaminated soils cause Zn phytotoxicity depending on soil pH and fertility.

Table 1. Estimated removal of Zn and Cd in crop biomass of maize (*Zea mays* L.) at full yield or 50% yield reduction due to Zn phytotoxicity and in biomass of *Noccaea caerulescens*, either the ‘Prayon’ race, or an improved cultivar derived from southern France populations with higher yield and 10-times higher Cd accumulation

Zn: Presume soil has 2000 ppm Zn=4000 kg Zn (ha•15 cm)$^{-1}$

Crop	Yield t DM ha^{-1}	Zn in Crop			Zn in Ash %
		mg kg^{-1}	kg ha^{-1}	% soil Zn	
Corn (normal)	20	50	1	0.0025	0.5
Corn (Zn toxicity)	10	500	5	0.0125	0.5
Noccaea	2.5	25000	61.2	1.53	40
Noccaea (improved)	5	25000	125	3.12	40

Cd: Presume soil has 20 ppm Cd = 40 kg Cd (ha•15 cm)$^{-1}$

Crop	Yield t DM ha^{-1}	Cd in Crop			Cd in Ash %
		mg kg^{-1}	kg ha^{-1}	% soil Cd	
Corn	20	0.1	0.002	<0.01	0.0002
Corn (Zn toxicity)	10	5	0.05	0.125	0.005
Noccaea (‘Prayon’)	2.5	200	0.5	1.25	0.4
Noccaea (South France)	2.5	2000	5	12.5	4
Noccaea (Improved)	5	2000	10	25	4

Table 1 shows the concentrations of Zn and Cd in a high biomass forage crop, maize (*Zea mays* L.) compared with the hyperaccumulator *Noccaea* (formerly *Thlaspi*) *caerulescens* (J. & C. Presl) F.K. Meyer (Brassicaceae). Maize forage grown on a normal uncontaminated soil contains about 25 mg Zn kg^{-1} dry matter (DM) and 0.10 mg Cd kg^{-1} DM. If the crop is grown on a contaminated soil with normal ratio of Cd:Zn from smelter or mine waste contamination [about 1 g Cd (200 g Zn)$^{-1}$], it will suffer significant Zn phytotoxicity at about 500 mg Zn kg^{-1} shoot DM (Chaney, 1993), and because of the relationship between Cd and Zn in uptake by maize and most other plant species, only about 5 mg Cd kg^{-1} DM can be reached before Zn phytotoxicity limits Cd phytoextraction. It is evident from Table 1 that a 50% yield-reduced maize crop suffering significant Zn phytotoxicity can only remove a very small amount of Zn and a trivial amount of Cd. No crop plant can do appreciably better in annual removal of Zn or Cd because Zn will reduce yields starting at about 400-500 mg kg^{-1} shoot DM (Chaney, 2010).

The Zn hyperaccumulator *N. caerulescens*, on the other hand, can grow well with up to 25,000 mg Zn kg^{-1} DM; individuals of a southern France race can accumulate 2500 mg Cd kg^{-1} DM from the same soil with normal geogenic Cd:Zn ratio. The original ‘Prayon’ population from Belgium (studied by many researchers) can accumulate only low levels of Cd compared to southern France plants (Chaney et al., 2000; 2010; Reeves et al., 2001; Schwartz et al., 2006). With the southern France races, annual removal of Cd can be enough to achieve a phytoremediation technology. In addition, lowering soil pH to near 5.5 can significantly increase Cd and Zn accumulation in *N. caerulescens* and hasten phytoextraction of soil Cd (Simmons et al., 2014; Wang et al., 2006; Yanai et al., 2006). Combining improved cultivars of Cd hyperaccumulators with improved agronomy to produce the crop with highest attainable yield of Cd in shoot biomass offers soil Cd remediation at much lower cost than removal and replacement of the surface tillage depth of soil, which is generally considered to cost about US$1 million ha^{-1}. Iwamoto (1999) described engineering remediation of 498 ha of contaminated rice soils in the Jintzu Valley, Toyama, Japan at a cost of about US$2.5 million/ha.

As we have discussed previously, areas of Cd+Zn contaminated rice paddy soils in Asia have caused human Cd disease in at least Japan, China, Thailand, and Vietnam (Chaney et al., 2004; 2007a; 2013). This occurs because rice (*Oryza sativa* L.; Poaceae) is traditionally grown in flooded soils. When fields are drained at flowering, soil Cd can be rapidly converted to phytoavailable forms and soil pH can drop to low levels which favor Cd uptake. Further, polished rice grain is deficient in Ca, Fe, and Zn for human nutrition and these deficiencies cause up to 10-fold higher absorption of Cd by humans. In mammals Cd is mostly absorbed on the DMT1 Fe^{2+} transporter in the duodenum such that deficiency of Fe and Zn strongly increase Cd absorption (see Reeves & Chaney, 2008; Chaney et al., 2013). Recent research has shown that most Cd absorbed by rice roots is transported on NRAMP5 (Ishikawa et al., 2012; Ishimaru et al., 2012; Sasaki et al., 2012), which is a Mn transporter, while in wheat and other crops Cd is accumulated on the Zn transporter such that high Zn strongly inhibits uptake of Cd when the normal geogenic Cd:Zn ratio occurs in soil (*e.g.*, Hart et al., 2005; McKenna et al., 1992). Radiation mutation and selection of the null mutant of NRAMP5 yielded a very low Cd rice genotype that can legally be grown on contaminated paddy soils. If rice is being grown on aerobic soils to reduce accumulation of inorganic As in grain, it is possible that the disabled NRAMP5 genotypes will suffer Mn deficiency. Separately, over-expression of the HMA3 gene increased pumping of Cd into root cell vacuoles and kept rice grain Cd at low concentrations (Ueno et al., 2010); but GMO cultivars are not allowed yet for rice (note that selection of ineffective HMA3 mutants (Murakami et al., 2009; Ueno et al., 2011) allows high Cd transport to rice shoots as discussed below). In addition, subsistence rice farm families consume rice 'home-grown' on their Cd-contaminated soils, leading to extreme Cd exposures. Because contaminated rice soils are responsible for essentially all demonstrated human Cd-disease caused by soil Cd, there is a large need for effective Cd phytoextraction technology.

Another crop which accumulates relatively high levels of Cd which can be accumulated by humans is tobacco (*Nicotiana tabacum* L.; Solanaceae). Tobacco accumulates Cd up to about 25 mg kg^{-1} DM in soils with 1 Cd:100 Zn contamination (such as from smelters, mine wastes, or historic biosolids) before Zn phytotoxicity strongly reduces yield. For example, normal high yielding tobacco crops accumulated 17 mg Cd kg^{-1} DM when grown in a field with mine waste contamination in China (Cai et al., 1990), over 11-25 mg kg^{-1} DM in Pb- and Zn-smelter contaminated soils in Bulgaria (Angelova et al., 2004; Chuldjian & Chaney, unpublished) and up to 70 mg kg^{-1} DM when grown on acidic soils treated with Cd rich biosolids (Mulchi et al., 1987). Tobacco contributes as much or more Cd to the kidneys of smokers than all of their dietary crop foods. Some of the tobacco Cd enters the mainstream smoke and is very effectively absorbed in the lung. It is generally estimated that smoking normal cigarettes with 1 mg Cd kg^{-1} DM at one pack per day from age 20 to 50 doubles the Cd concentration in kidney cortex (Elinder et al., 1976). Thus, both rice and tobacco soils mineralized or contaminated with Cd require remediation (or change in crop grown) to protect human health.

Cd-phytoextraction technology has been sought for rice paddy soils by several research groups. Studies in Japan largely confirmed that most crop plants cannot remove enough Cd to achieve useful phytoextraction (Ishikawa et al., 2006). Ishikawa et al. (2006) clearly show that *Brassica juncea* Czern. (Brassicaceae) has no practical value in phytoextraction because it is not a hyperaccumulator and is not tolerant of accumulated metals. Unfortunately, *N. caerulescens* is not adapted to the tropical climate of these fields. Until the study by Simmons et al. (2014), the use of *N. caerulescens* to remove Cd from rice soils had not been successful. Simmons et al. (2014) found that improving soil drainage by ridge planting, acidification, and application of fungicides allowed survival and effective growth and Cd phytoextraction by southern France genotypes growing in a Thai tropical setting. Alternatively, unusual rice cultivars with an ineffective HMA3 gene for storage of Cd in root vacuoles transport enough Cd to shoots that lowering soil pH and growing a high yield of shoots with up to 100 mg Cd kg^{-1} DM offers a valid phytoextraction technology (Murakami et al., 2009). In contrast to major contaminating metals such as Zn and Pb, Cd concentrations are usually relatively low so that acidifying the soil to near pH 5.5 to obtain rapid annual removal of several kg Cd ha^{-1} and then returning the pH to >6.5 might reduce crop Cd to acceptable levels. With the recognition that perhaps 40,000 ha of land in Japan, and likely more than that in China, require Cd remediation to produce rice which meets the CODEX international limit of 0.4 mg Cd kg^{-1} FW, there is renewed interest in commercial Cd phytoextraction. These estimates of rice land in need of Cd-phytoextraction could grow substantially if rice will need to be produced on aerobic soils to reduce grain levels of As (Zhao et al., 2010).

Other soil Cd contamination cases may also benefit from phytoextraction: these include soils with high Cd:Zn ratio from biosolids, soil contaminated by Cd industries, and some Cd-mineralized marine shale-derived soils with geogenic Cd high enough to cause excessive crop Cd. In these cases other plant species may also be useful. With high rates of metal-rich biosolids, soils may be rich in phytoavailable Cu which can limit *N. caerulescens* growth (McLaughlin & Henderson, 1999). Schwartz et al. (2003) studied Cd phytoextraction from field plots in France where high Cd biosolids had been applied and showed that *N. caerulescens* could significantly decrease Cd accumulation by lettuce (*Lactuca sativa* L.; Asteraceae) post-phytoextraction. Broadhurst et al. (2014) tested a maize "inbred" with unusually strong Cd accumulation which grew well on high metal biosolids-amended soil and appears to offer Cd phytoextraction capability similar to HMA3 mutant rice genotypes. No other plant species has been shown to provide this capability for Cd rich soils with simultaneous high Cu levels and, as noted above, most species claimed to be Cd hyperaccumulators based on spiked soil or nutrient solution tests with Cd salt addition without Zn are not of any use in practical phytoextraction of contaminated soils (van der Ent et al., 2013).

Another approach suggested by some is growth of bioenergy crops such as willow (*Salix* spp.) or maize with comparatively high Cd accumulation and high yield ability compared to most crop plants, but not nearly a Cd hyperaccumulator (*e.g.*, Thewys et al., 2010; Witters et al., 2012). If the bioenergy crop paid for the Cd phytoextraction practice over a long period and the ash or other residue is placed in landfills, it might be a cost-effective alternative for phytoextraction (Thewys et al., 2010). This group conducted "Life Cycle Assessment" to estimate the time required for Cd removal to allow production of vegetable crops.

Several *Sedum* species with true Cd hyperaccumulator ability (*Sedum alfredii* Hance, *Sedum plumbizincicola* X.H. Guo et S.B. Zhou ex L.H. Wu, and *Sedum jinianum* X.H. Guo; Crassulaceae) have been identified in China (Deng et al., 2007; Liu et al., 2011; Wu et al., 2013; Xu et al., 2009; Yang et al., 2004), but none exhibit the extreme Cd accumulation of southern France *N. caerulescens*. These species are taller than *N. caerulescens* and appear to offer higher harvestable annual yields. Several other natural Cd hyperaccumulators which accumulated over 500 mg Cd kg^{-1} DM in the field have been found at a tropical Zn-Cd mine site in Thailand (Phaenark et al., 2009). Study of these species has been limited because they were reproduced only by cuttings. One of the species (*Gynura pseudochina* (L.) DC.; Asteraceae) found was 0.4-1 m tall and accumulated 458 mg Cd kg^{-1} and 6.17 g Zn kg^{-1} DW on a soil with 184 mg Cd kg^{-1} and 16.7 g Zn kg^{-1}, and grew well in the rainy season (this had limited the yield of *N. caerulescens*). Thus the shoot biomass contained a much higher Cd:Zn ratio than the soil, similar to southern France *N. caerulescens*. Khaokaew et al. (2014) confirmed the value of this species for Cd phytoextraction in contaminated rice fields. A tropical fruit tree, carambola (*Averrhoa carambola* L.; Oxalidaceae) or star fruit, which accumulates relatively high levels of Cd has been identified by Li et al. (2010; 2011). Use would require high planting density and rapid planting because the seeds have a very short lifetime after harvest of the fruit. Because of its tropical adaptation, research is continuing to develop this species for practical Cd phytoextraction. Additional searching for natural strong Cd accumulators for tropical soils is needed.

Many scientists have ignored the fundamental definition of hyperaccumulators: the accumulation of an element above some limit for a plant growing in soils where the species occurs naturally (van der Ent et al., 2013); and the usual 100-200 times higher soil Zn than Cd in geological Zn+Cd enrichment. If Zn kills crops with about 500 mg Zn kg^{-1} DM, and a species accumulates Cd and Zn at about the ratio in soil, the plant will reach no higher than 5 mg Cd kg^{-1} DM. Growing plants in Cd-salt spiked soils or nutrient solutions with Cd addition does not test their utility for phytoextraction, and over 10 species have been claimed to be hyperaccumulators of Cd based on this false definition.

OTHER PHYTOEXTRACTION TECHNOLOGIES TO PROTECT HUMAN HEALTH

Excessive soil Se has long harmed livestock and wildlife, and human Se toxicity has been observed in China from food crops (Yang et al., 1983). Phytoextraction of soil Se from such soils is also needed to protect the safety of irrigation drainage waters which may harm wildlife (Bañuelos et al., 1997). It is also possible that increased Se in foods could contribute to improved human health, and Se-rich crops might be sold as ‘nutraceuticals’ (Bañuelos & Dhillon, 2011) or used to replace mined Se salts in livestock feeds (Bañuelos & Mayland, 2000). Interestingly, the ability of the natural Se hyperaccumulators to accumulate high levels of Se in the presence of high levels of soil sulfate is a critical part of the Se hyperaccumulator characteristic (Bell et al., 1992). In the phytoextraction model of Bañuelos & Dhillon (2011), use of several relatively high Se-accumulating crop plants can be a safe practice because sulfate limits Se accumulation to levels which will not be harmful in foods, but gives significant removals from a mineralized or contaminated soil to limit Se risk from irrigation drainage waters.

Soil As can be high from both mineralization and contamination. Most plants accumulate only low levels of As (Zhao et al., 2010), but the fern *Pteris vittata* L. (Pteridaceae) was found to accumulate high levels of As on slightly contaminated soils (Ma et al., 2001). Recent recognition that rice grown in flooded soils commonly accumulated high levels of possibly carcinogenic inorganic As in grain and stover has raised questions about the safety of rice and rice products. Growing rice in aerobic soils strongly reduces grain As but lowers yield, so extensive effort will be required to breed genotypes of rice adapted to aerobic soils which give both high grain yields and quality along with low grain levels of inorganic As and Cd. *Pteris vittata* is a tropical species which can be grown in aerobic rice soils. Several fern species are being tested by research groups to learn if phytoextraction can remove enough As to allow production of rice grain with lower As levels, but no successful field demonstration has yet been reported.

Induced Phytoextraction

Another proposed phytotechnology is 'induced phytoextraction,' in which chelating agents are applied to soils to dissolve soil metals and aid their uptake by plants (*e.g.*, Blaylock et al., 1997). As discussed previously by Chaney et al. (2010), addition of chelating agents to promote plant uptake of soil metals is neither cost effective nor environmentally acceptable. Nowack et al. (2006) provides a thorough review of the environmental risks of using chelating agents to induce phytoextraction: ultimately, more metals are leached than are absorbed by plants. We obtained information to make a new estimate of the cost of applying EDTA for induced phytoextraction. We assume 10 mmol Na_2EDTA kg^{-1} soil and that the EDTA is purchased in truckload (20 t) quantities. The price of technical grade $Na_2EDTA{\bullet}2H_2O$ (FW 372 g mol^{-1}) (US\$3.16 kg^{-1} in 2014) was obtained from a major international manufacturer. Assuming 15 cm depth of soil Pb contamination with $2{\bullet}10^6$ kg soil ha^{-1}, one application of Na_2EDTA at 10 mmol kg^{-1} soil costs US\$23,500 ha^{-1}. Induced phytoextraction with EDTA was never a good idea and has not been permitted for over 10 years in the US or the EU.

For gold, application of cyanide or thiocyanate to soils can promote plant uptake, but this cannot be done in open environments, only on leaching pads with plastic liners to collect and treat any leachate (Anderson et al., 2005). A similar result has been reported for Hg contaminated soils where application of thiosulfate may allow significant phytoextraction of soil Hg (Pedron et al., 2013). Although Hg phytovolatilization has been developed (Heaton et al., 1998), emission of soil Hg to the global pool of atmospheric Hg has not become a favored technology.

Phytomining

The issues and concepts involved in phytomining are presented below, with focus on Ni. Ni is the element for which phytomining appears most feasible because of the widespread occurrence and extent of Ni-rich ultramafic soils and mine wastes, the wide variety of Ni-accumulating plants, and the ready market for Ni metal, Ni salts and Ni fertilizers. However, the process is much more complex than simply finding a suitable tract of land and growing hyperaccumulator plants.

The central rationale for Ni phytomining is that the Ni concentration of currently mined ore materials is typically 0.8-2.5% Ni, whereas the Ni concentration of certain hyperaccumulator plants is 1-3% Ni in dry leaf tissue (or 8-25% in plant ash). These plants grow on soils with 0.05-0.8% Ni, which would be sub-economic for mining, but the plant ash constitutes an ore material an order of magnitude richer than mined ores. Further, the plant ash is low in Fe and Mn oxides and Mg silicate which make recovery of Ni from lateritic ores complicated and expensive.

A question may be asked about the long-term sustainability of the phytomining process. Over an area with Ni averaging 2500 mg/kg to 30 cm rooting depth, the total Ni present is about 10 t Ni (ha-30 cm)$^{-1}$ (Table 2). A single crop of a hyperaccumulator plant with dry weight 10 t ha^{-1} and 2% Ni yields 200 kg Ni ha^{-1}, which is 2% of this resource. Thus phytomining of the area should be sustainable on a 50-year time scale if the soil/subsoil is turned over periodically and if natural buffering processes replenish the plant-available Ni on the time scale of the phytoextraction process. It is likely that for the same site and pH, Ni concentration in phytomining crop shoots will decline over time as the readily phytoavailable pool is depleted. This condition is site-specific. We infer that Alyssum (Brassicaceae) hyperaccumulators also obtain Ni from subsurface soils in serpentine soils because Ni accumulation is higher when these species are grown on serpentine soils than when they are grown on Ni-refinery contaminated soils with similar total Ni concentration where the Ni is limited to the tillage depth (Chaney, unpublished).

The following are the critical steps to consider in establishing a phytomining operation: (1) selection of a suitable land area; (2) selection of suitable plant species; (3) planting technology; (4) harvesting strategies; (5) post-harvest treatment of biomass and marketing; and (6) the economics of the whole process. Some of these factors have been discussed by various authors (Angle et al., 2001; Brooks & Robinson, 1998; Chaney et al., 2000; 2007a; 2010; Li et al., 2003a; b). Unfortunately, only some of the world's ultramafic areas are suitable for arable-style cropping of Ni hyperaccumulator plants, as many exposures have steep and/or rocky topography, and even some of the flatter land may be too stony for mechanical cultivation. Some adjacent colluvial soils with strong Ni enrichment could also be phytomined. Extensive preliminary soil analysis is required to establish the extent of the resource because usual soil survey reports showing soils derived from serpentine parent rocks have little relationship with soil Ni levels or phytoavailability, and adjacent Ni-rich colluvial soils are not recognized as potential Ni resources. The rainfall regime needs to be considered because the profitability of the process is reduced by any need for irrigation. Land ownership factors may add complexity to the arrangements, and long-term commitments of owners as farmers or as lessors of the land need to be established.

Table 2. Estimated Ni phytoextraction by maize (*Zea mays* L.) vs. *Alyssum murale* grown as a phytomining crop; assume control soil contains 25 mg Ni kg^{-1} and the Ni-rich soil contains 2500 mg Ni kg^{-1} = 10,000 kg Ni (ha•30 cm)$^{-1}$; assume soil Ni is sufficiently phytoavailable that corn has 50% yield reduction compared to corn grown on similar soil without Ni mineralization. Research has shown that unimproved *Alyssum murale* can easily yield 10 t ha^{-1} with fertilizers, and selected cultivars can exceed 20 g Ni kg^{-1} DM with appropriate soil and crop management on serpentine soils. Most crop plant species suffer ≥25% yield reduction when the shoots contain 100 mg Ni kg^{-1} dry weight. Ni concentration in ash is limited by formation of $NiCO_3$ with only 49% Ni

Crop	Soil	Yield t DM ha^{-1}	Ni in Crop			Ni in Ash %
			mg kg^{-1}	kg ha^{-1}	% soil Ni	
Maize	Control	20	1	0.02	0.01	0.002
Maize (50% Yield)	Ni-rich	10	100	1	0.01	0.2
Wild *Alyssum* in pasture	Ni-rich	3	10,000	30	0.3	10-15
Wild *Alyssum murale* + fertilizer	Ni-rich	10	20,000	200	2	20-25
Alyssum murale cultivar	Ni-rich	20	25,000	500	5	25-30

Selection of suitable plant species is also not straightforward. The most obvious criteria are the maximum (and typical) Ni concentrations so far found in the plant in its natural environment, and the annual yield of biomass. Experimental work can establish the possibility of enhancement of total uptake through appropriate fertilization. However, the suitability of plant species to the phytomined environment must be taken into account: this includes not only the need to ensure that the species is appropriate to the climatic conditions, but also the need for physical protection of the plants, protection against pests and diseases, and biosecurity issues. In this last respect, we note that many countries (particularly those not sharing a land border with another country) are increasingly strict about the introduction of new species, and even within a country there may be concern about the effects of transferring a species from one region to another. In view of the fact that many ultramafic areas host rare and/or endemic species, the maintenance of existing biodiversity in the face of the possible spread of the phytomining crop species becomes a serious concern. For several reasons, therefore, there are strong arguments in favor of using species native to the region (or sterile cultivars of non-natives) for any phytomining venture.

From more than 400 known Ni hyperaccumulators about 150 have shown Ni in leaves at concentrations that can exceed 1%; more than 70 of these are from Mediterranean-climate areas, and at least another 70 are from tropical areas. They include herbs (annual and biennial), shrubs (small and large; short- and long-lived perennial), and trees. In some species the high-Ni tissue is a low proportion of total biomass, rendering them less suitable as phytomining candidates.

The following are a few examples of Ni hyperaccumulator species that seem to have the best potential, some of which have already been the subjects of extensive field and pilot-scale trials. From more than 50 hyperaccumulators in the genus Alyssum, several species (e.g. *A. murale* Waldst. & Kit., *A. corsicum* Duby, *A. lesbiacum* (Cand.) Rech.f., and *A. pinifolium* (Nyár.) Dudley, among others, native to Turkey, Greece, and the Balkan region) are short-lived perennials that appear appropriate for areas with a Mediterranean-type climate. Another key property of these species is that their height and growth pattern are amendable to mechanical harvest. A few other Mediterranean members of the Brassicaceae may also be suitable, such as *Leptoplax emarginata* (Boiss.) O.E. Schulz (Greece), *Bornmuellera* species (Greece and Turkey) (e.g., Cai et al., 2005), and the largest of the species of Thlaspi such as *T. jaubertii* Hedge (Turkey). In the Asteraceae, useful biomass is also produced by some hyperaccumulator species in *Centaurea* in Turkey (e.g., *C. ptosimopappa* Hayek, *C. ensiformis* P.H. Davis) (Reeves & Adıgüzel, 2008) and by species of *Berkheya* and *Senecio* in South Africa (Morrey et al., 1992). Detailed discussion of experiments relevant to phytomining with *Berkheya coddii* Roessl. and the Italian *Alyssum bertolonii* Desv. has been given by Brooks & Robinson (1998) and Brooks et al. (2001).

Although a list of tropical Ni hyperaccumulators with >1% Ni has been published (Reeves, 2003), they have generally been less intensively studied for their phytomining potential. In tropical regions the largest resources of Ni hyperaccumulators come from the ultramafic floras of Cuba and New Caledonia, although additional species from areas of Indonesia, Philippines, and Malaysia (Sabah) should also be considered. Examples include large shrub or small tree species such as *Rinorea bengalensis* (Wall.) O.K. (Violaceae) and *Dichapetalum gelonioides* (Bedd.) Engl. subsp. *tuberculatum* Leenh. (Dichapetalaceae) from SE Asia, Geissois (Cunoniaceae) species from New Caledonia, and species from the very large genera *Phyllanthus* (Euphorbiaceae) and *Psychotria* (Rubiaceae; Cuba, New Caledonia, S.E. Asia). In many cases we still have insufficient knowledge about important matters for these species such as rates of biomass production and efficient methods of propagation.

Experimental work is needed to optimize planting technology and to compare seed drilling with the planting out of seedlings or the production of rooted cuttings (for shrub species). Planting densities need to be optimized, e.g., by use of Nelder plots (Angle et al., 2001), to achieve the maximum biomass production. Harvest strategies include the harvest of the total aboveground biomass of annual herbs or short-lived small shrubs, renewable harvest of annual growth of perennial shrubs, coppicing of large shrubs or small trees, or the total harvest of large trees.

Decisions also need to be made regarding the post-harvest treatment of biomass. Options include natural or energy-assisted drying, followed by ashing or chemical digestion. Where ashing is chosen, there is the possibility of energy recovery from the biomass combustion. Ash may be transported to be used as feedstock for a conventional smelter or a stand-alone extraction process may be developed, in which the Ni is extracted from unashed or ashed biomass by chemical and/or electrochemical means. Treating Ni-rich ash as a smelter additive appears to be the simplest way to proceed (Chaney et al., 2007a), as it is unlikely that in any one area an operation producing about 200 kg Ni ha^{-1} yr^{-1} could be conducted on such a scale as to make stand-alone ash-processing equipment economically viable.

Ultimately, the economics of the whole process must take into account: (1) the total development and optimization costs through pilot plant stages to full operation; (2) the annual costs of maintaining the process (labor and machinery costs of propagation and harvesting, fertilizer and irrigation if needed, and plant protection measures); and (3) land lease costs, if any. The final analysis is highly dependent on the rather volatile price of Ni on world markets, and on the net value of alternative uses of the land (e.g., for forestry or for grain crops), which can themselves be subject to considerable price fluctuations.

Wheat (*Triticum aestivum* L.; Poaceae) yields in the USA average about 50 bushels per acre or 3.3 t ha^{-1}; prices for the harvested grain crop since 1998 have ranged from about US$2-9 per bushel, depending on the season and the incidence of natural disasters such as flood, storm, and drought. The returns therefore correspond to about US$224-1008 ha^{-1}. For wheat grown on serpentine soils the yield would be suboptimum and the returns would be at the lower end of this range. Recently Ni has sold for about $16-20 kg^{-1}, such that growing a good crop of *Alyssum* (10 t ha^{-1} dry matter), which at 2% Ni contains about 200 kg Ni, would be valued at US$3200-4000 after processing. This indicates the economic viability of Ni phytomining, at least in the situation where processing costs (transport, ashing, refining) are low; this should be the case if the plant ash is treated as a minor but Ni-rich feedstock for a larger-scale conventional smelter operation.

Chaney et al. (1998; 1999; 2007b) obtained patents (expiring in 2015) for practical Ni phytomining using *Alyssum murale*, *A. corsicum*, and other high Ni accumulating species, and reported that *Alyssum* ash was an excellent ore when processed by electric arc furnace (Chaney et al., 2007a; 2010). Bani et al. (2014) tested methods for phytomining of Ni in Albanian serpentine soils starting with collecting wild plants, then adding fertilizer to wild plants, then applying both fertilizer and herbicides to wild plants, and finally preparing the soil and planting seeds of several Ni hyperaccumulators with phytoextraction yields over 100 kg ha^{-1}. Li et al. (2003b) reported higher Ni concentrations and Ni yields when growing improved cultivars of *Alyssum* generated in our breeding program.

The group of Morel et al. (Barbaroux et al., 2012) also attempted to produce $Ni(NH_4)_2(SO_4)_2$ as a more valuable commercial Ni chemical from the biomass ash rather than only Ni metal. Possible Ni products which could be made from hyperaccumulator biomass ash besides Ni metal will require additional experimentation (Hunt et al., 2014). A minor use for Ni-rich biomass is as an ‘organic’ Ni fertilizer in remedying plant Ni deficiency, known to occur in pecan trees (Wood et al., 2005; 2006).

Some predicted that Ni availability to hyperaccumulators would follow the patterns known for crop plants, and that the Ni extractable by usual agricultural extractants such as 1.0 M NH_4-acetate would be related to uptake such that extractions with this reagent could predict the utility of Ni phytomining (Robinson et al., 1999a; b). With this extractant, lowering soil pH increases extractable Ni (Figure 1). However, in our studies of agronomic factors in Alyssum Ni phytomining, we learned that lowering pH increased extractable Ni but actually reduced Ni accumulation in *Alyssum* (Kukier et al., 2004; Li et al., 2003a). Further, it has been clearly shown that *Alyssum* hyperaccumulators extract Ni from the same soil labile Ni pool do as other plant species (Massoura et al., 2004). The pH response on *Alyssum* Ni accumulation is illustrated in Figure 2 which shows that lower pH usually reduces Ni concentration in shoots of *Alyssum* species (a similar pH response was observed for *B. coddii* Ni hyperaccumulation; Chaney et al., unpublished). For soils with very high levels of Fe oxides, raising pH above 6.2 may reduce Ni accumulation because of the stronger binding and occlusion of Ni by Fe oxides at higher pH (Kukier et al., 2004). These data were selected from our study of the effect of soil acidification on Ni hyperaccumulation by *Alyssum* species from 20 Ni-rich smelter contaminated or serpentine soils. Robinson et al. (1999b) reported that for Ni hyperaccumulators, adding chelating agents to the soil actually decreased uptake of Ni in contrast with the use of EDTA to promote Pb uptake noted above.

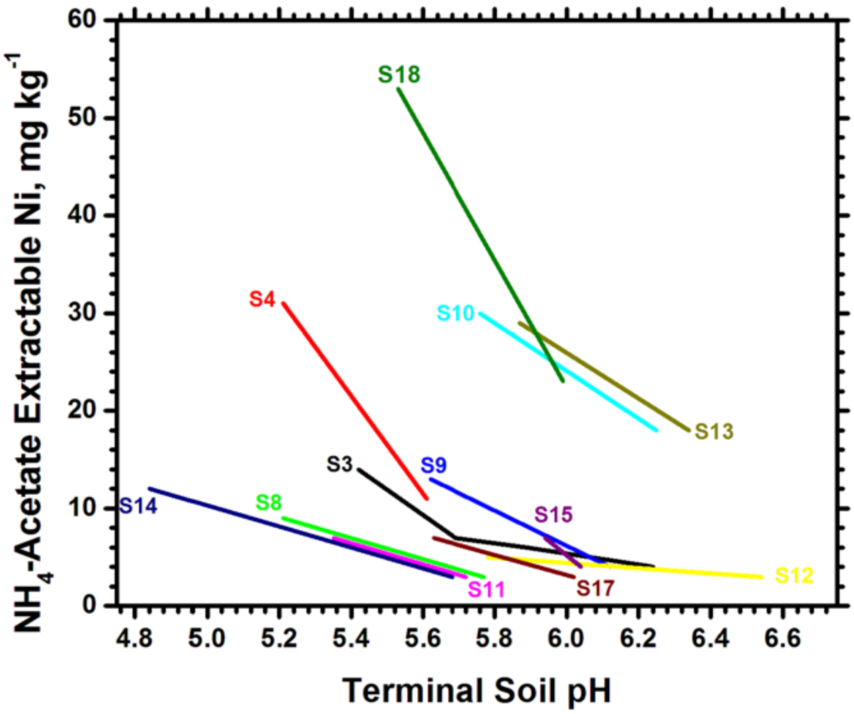

Figure 1. Increase in soil pH decreases 1 M NH_4-acetate extractable Ni in diverse serpentine soils (Chaney et al., unpublished; Li et al., 2003a).

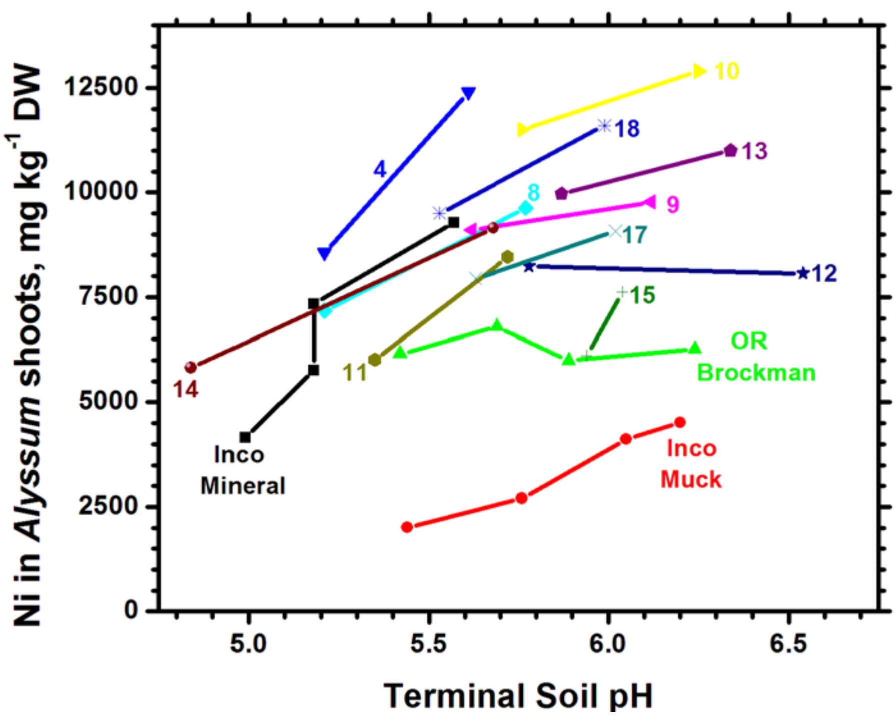

Figure 2. Increasing soil pH increases Ni accumulation by *Alyssum* species grown on serpentine and Ni refinery contaminated soils (Chaney et al., unpublished; Li et al., 2003a).

Several research groups have tested the effect of soil microbes on Ni hyperaccumulation. Abou-Shanab et al. (2003) found that some strains of rhizosphere bacteria could increase yield of *A. murale* shoot Ni even when inoculated into non-sterilized serpentine soil. Cabello-Conejo et al. (2014) reported similar results for *A. pintodasilvae*. Orłowska et al. (2011) reported that various mycorrhiza increased Ni yield in *Berkheya coddii* Dudley grown on a serpentine soil. *Berkheya coddii* was found to have mycorrhiza symbioses in the field by Turnau & Mesjasz-Przybylowicz (2003) which suggests that the relatively low Ni accumulation by this species in studies in soils away from South Africa may have been a result of missing a specific mycorrhizal fungus from *Berkheya*'s native soils. Brassica species do not support mycorrhizae, so the *Alyssum* species reaction with mycorrhizae have not been reported.

It is also conceivable that 'organic' B fertilizers could be produced by phytoextraction on B phytotoxic soils by harvesting B-rich leaves (e.g., Robinson et al., 2007). Anderson et al. (1999) estimated that Tl could be economically phytomined, and plant species with excellent Tl accumulation and useful yields have been identified (see also LaCoste et al., 1999). No commercial Tl phytoextraction has been reported to date.

Cobalt phytoextraction is theoretically profitable, but no technology has been identified to date. Malik et al. (2000) compared Co accumulation by several species known to accumulate Co. Their work showed that Ni in serpentine soils inhibited Co accumulation by *Alyssum* species, and that optimal Co accumulation occurred at low pH while that of Ni occurred at higher pH (Kukier et al., 2004). Thus one model for Ni and Co phytomining is to maximize Ni removals at higher pH, and then acidify soil to phytomine soil Co. It is also conceivable that land with ^{60}Co contamination could be phytoextracted to remove the radionuclide at far lower cost than removal and hauling of the soil to a radionuclide landfill. The long half-life of ^{60}Co (5.3 yr) does not encourage simply waiting for decay of this radionuclide. Unexpectedly, Tappero et al. (2007) found that although both Co and Ni were accumulated by a root transporter, and pumped into the xylem by another transporter, Co was not transported into epidermal cell vacuoles along with Ni. Cobalt was not accumulated in leaf epidermal cells of *Alyssum* species, but was precipitated outside cells at the end of leaf veins.

As noted in Table 3, the Cu-Co accumulators of central Africa are no longer believed to usefully hyperaccumulate these elements (Faucon et al., 2007), but the plants continue to be excellent bio-indicator or botanical-prospecting plants for Cu-Co deposits (e.g., de Plaen et al., 1982). Other Cu accumulators may reach the newly accepted Cu hyperaccumulator limit of 300 mg kg^{-1} (van der Ent et al., 2013), but they are not useful for phytoextraction of soil Cu because the annual Cu removal would be very small compared to the levels of Cu in contaminated soils needing remediation (Faucon et al., 2009; Kolbas et al., 2014; Peng et al., 2012).

Table 3. A selection of plant species which hyperaccumulate elements to over 1% of their shoot dry matter; usually to at least 100-fold levels tolerated by crop species

Element	Plant species	Maximum metal concentration mg kg^{-1} dry wt.	Location collected	Reference
Zn	*Noccaea caerulescens*	39,600	Germany	Reeves & Brooks, 1983a
Cd	*Noccaea caerulescens*	2,910	France	Reeves et al., 2001
Cu[1]	*Aeolanthus biformifolius*	13,700	Zaire	Brooks et al., 1978
Ni	*Phyllanthus serpentinus*	38,100	New Caledonia	Kersten et al., 1979
Co[1]	*Haumaniastrum robertii*	10,200	Zaire	Brooks et al., 1978
Se	*Astragalus racemosus*	14,900	Wyoming, USA	Beath et al., 1937
Mn	*Alyxia rubricaulis*	11,500	New Caledonia	Brooks et al., 1981
As	*Pteris vittata*	22,300	Florida, USA	Ma et al., 2001
Tl	*Biscutella laevigata*	15,200	France	Anderson et al., 1999

[1]Although Cu and Co hyperaccumulation were confirmed in field collected samples, similar concentrations have not been attained in controlled studies and additional research showed that much of the shoot Cu and Co was from soil contamination (Faucon et al., 2007; 2009).

PHYTOSTABILIZATION OF METAL CONTAMINATED SOILS

Zinc-, Cu-, Ni- and Pb-contaminated soils occur in many countries and locations. Because of co-mineralization of Zn-Pb and Zn-Cu ores, Zn-Cd-Pb mixed contamination occurs at literally thousands of locations. If these are alkaline, infertility is the main limitation, although Pb risk through soil ingestion may be important. However, because Zn is usually present at 100-200 times higher concentration than Cd, and Zn in a crop reduces the bioavailability of Cd in a crop, few locations pose Cd risk except through rice and tobacco as discussed above. Thus phytostabilization of soil Zn and Pb might allow cost-effective remediation of contaminated soils and protect the environment. Some mine wastes or smelter contaminated sites have become extremely acidic from co-occurring pyrite so that high Zn and pH below 4.5 occur together and prevent growth of nearly all plant species (*e.g.,* Beyer et al., 2010; Brown et al., 2003b; Li et al., 2000). It is possible to grow some metal-tolerant ecotypes as discussed above, such as the grasses *Agrostis capillaris* L. (Poaceae) or 'Merlin' red fescue (*Festuca rubra* L.; Poaceae) developed by Smith & Bradshaw (1972), or Ni- and Zn-tolerant *Deschampsia cespitosa* (L.) P. Beauv. (Poaceae; Cox & Hutchinson, 1980; von Frenckell-Insam & Hutchinson, 1993).

Phytostabilization will usually require incorporation of soil amendments to reduce metal solubility/phytotoxicity (liming), addition of metal sorbents (Fe and Mn oxides), and addition of organic matter with soil microbes (composts or biosolids), along with any other fertilizers needed to reduce soil metal bioavailability or satisfy plant nutrient requirements, and so attain revitalization (Allen et al., 2007; Brown et al., 2005; Chaney et al., 2010; Stuczynski et al., 2007). Soil Pb contamination occurs widely and causes risk to very young children, especially in urban and garden soils, because soil can be carried into homes where very young children might ingest Pb-rich housedusts by hand-to-mouth transfer (Ryan et al., 2004; Scheckel et al., 2013; Zia et al., 2011). The number of children at risk from Pb in mine wastes is trivial compared to those exposed to urban Pb-rich soils from historical automotive exhaust and paint Pb contamination (Scheckel et al., 2013; Zia et al., 2011). Fortunately, addition of phosphate can promote the formation of chloropyromorphite [$Pb_5(PO_4)_3Cl$] which has been shown to have low bioavailability. Ryan et al. (2004) conducted a field test using several P-rich soil amendments to reduce the bioavailability of soil Pb and fed the treated soils to rats, pigs, and humans. The experiment showed that high phosphate application could reduce soil Pb bioavailability to rats, pigs, and humans by nearly 70%. The soil had about 3000 mg Pb kg^{-1}, along with high levels of Zn and corresponding Cd. Making the soil pH neutral prevented any adverse effects of the Zn and Cd, and limited plant accumulation of all three elements (Brown et al., 2004). Further, Scheckel & Ryan (2004) showed that pyromorphite was indeed formed in phosphate treated soils. Besides phosphate-induced Pb inactivation, high Fe biosolids rich in phosphate have also strongly reduced soil Pb bioavailability to animals and Pb bioaccessibility (Brown et al., 2003a; 2004). A comprehensive review of soil Pb risk reduction by formation of chloropyromorphite has recently been published (Scheckel et al., 2013). The most important application of *in situ* Pb inactivation, or soil Pb phytostabilization, is for Pb-rich urban soils. Garden and yard soils are carried into homes on clothing and gardening equipment and become part of the house dust to which young children are exposed. Although interior paint is still the more important source of Pb poisoning of young children, concern about Pb in urban soils has raised the need for extensive phytostabilization of urban soil Pb (Scheckel et al., 2013; Zia et al., 2011).

Phytostabilization of Soil Ni

Smelter emissions and mine wastes in many locations have caused severe Ni phytotoxicity, preventing growth of most plant species. Sulfidic Ni-mine wastes generate strong acidity, which increases Ni^{2+} solubility and phytotoxicity. Large areas with Ni smelter deposition were denuded by the Ni toxicity mixed with SO_2 emissions where Ni deposition occurred in strongly acidic boreal forest areas of Canada (Amiro & Courtin, 1981; Courtin, 1994; Freedman & Hutchinson, 1980; Hutchinson & Whitby, 1977), Russia (Chernenkova & Kuperman, 1999; Helmisaari et al., 1999; Kozlov, 2005; Stjernquist, et al., 1998), and Norway and Finland (Almas et al., 1995). SO_2 caused acute toxicity and killed trees, forest fires often followed, and erosion caused loss of nutrients and organic matter. When SO_2 emissions were controlled to prevent the acute toxicity, soil Ni had accumulated to high enough levels in the very strongly acidic forest soils to prevent regrowth of most species. Highly Ni-tolerant grass ecotypes were selected at these locations (Cox & Hutchinson, 1980) and tree species which coppiced might persist.

Remediation of very strongly acidic, Ni-contaminated soils near smelters has been achieved through phytostabilization. The amount of limestone required depends on soil properties and pH, and dolomitic limestone was more effective than calcitic limestone at Sudbury apparently because both Ca and Mg were leached from the extremely acidic soils after sulfuric acid deposition (Lautenbach, 1987; Winterhalder, 1983). The extensive denuded area surrounding the Sudbury smelters was successfully remediated (phytostabilized) with limestone, fertilizer, and seeding (Gunn et al., 1995; Winterhalder, 1983; 1996). In the project at Sudbury, workers spread soil amendments and seeds on hilly soils to achieve the needed coverage. In general, surface applied limestone can increase pH in deeper soil only over periods of decades due to the slow diffusion of Ca. However, in several case studies surface application of high rates of combinations of organic and alkaline amendments has achieved the needed revitalization of contaminated soils (Brown et al., 2003b; Chaney et al., 2011; Stuczynski et al., 2007; Winterhalder, 1983). It is difficult or impossible to incorporate soil amendments on sloping soils or in forested soils, so leaching of alkalinity (Brown et al., 2003a) into soil profiles is necessary to make phytostabilization successful in such locations. Mixtures of biodegradable organic matter and alkaline materials allowed leaching of alkalinity down the profile. In the case of an asbestos mine waste site in Vermont, USA, the extreme Ca deficiency without Ni phytotoxicity was remediated using surface applied gypsum which yields leachable Ca to correct the extreme Ca deficiency of these serpentinite mine wastes (Chaney et al., 2011).

At another location in Canada, Ni refinery emissions were deposited on neutral to calcareous regional soils near Port Colborne, Ontario. In a small part of this contaminated area, Ni accumulated in acidic muck soils (to 2000 to 4000 mg Ni kg^{-1}) used for vegetable production and caused moderate and then severe Ni phytotoxicity to numerous vegetable crops, but not maize which is much more resistant to soluble soil Ni (Frank et al., 1982; Kukier & Chaney, 2004; McIlveen & Negusanti, 1994). The Ni toxicity of these soils was readily remediated by added limestone; but in the muck soils liming the soil reduced the phytoavailability of soil Mn and induced Mn deficiency, which caused the earlier researchers to believe that liming could not cure the Ni phytotoxicity of these soils. Adding Mn fertilizer with the limestone gave full remediation of these low Mn muck soils (Siebielec et al., 2007).

The volume and area of mine or ore beneficiation tailings at the Sudbury smelters grew over time and remained barren due to infertility and strong soil acidity from sulfide oxidation which increased Ni solubility and phytotoxicity (e.g., Bagatto & Shorthouse, 1999). Fertilization and limestone application achieved revegetation at these sites.

Although laterite and other ultramafic soils can be quite high in Ni, most such sites have limited plant species cover due to very low soil Ca:Mg ratio and low soil P status. These soil conditions cause long-term evolutionary processes which have selected serpentine tolerant species and ecotypes. Because most natural serpentine soils are near neutral pH and contain high levels of Fe oxides, most are not actively Ni phytotoxic (*e.g.*, Zhang et al., 2007). However, if soil pH drops due to soil formation processes or from use of acidifying N-fertilizers, Ni phytotoxicity can also occur in serpentine soils (Anderson et al., 1973; Crooke, 1956; Halstead, 1968; Hunter & Vergnano, 1952). Furthermore, if these soils are fertilized enough, crop plants can be grown. Oat (*Avena sativa* L.; Poaceae) grown on acidic serpentine soils shows Ni phytotoxicity via characteristic banded chlorosis (Hunter & Vergnano, 1952). Liming and addition of any other required nutrients (N, P, K, Mo, and/or B) can prevent Ni phytotoxicity of such Ni-contaminated or -mineralized soils (Kukier & Chaney, 2001; Siebielec et al., 2007) for all plant species (Kukier & Chaney, 2004). The early experiment by Crooke (1956) showed that addition of Na_2CO_3 was as effective as $CaCO_3$ in reversing Ni phytotoxicity in oat growing on acidic colluvial serpentine soil (not deficient in Ca).

Another case of mine waste phytostabilization has been demonstrated for disturbed asbestos mine wastes rich in Ni (>2,000 mg kg^{-1}). These mine wastes are essentially ground serpentinite rock with alkaline pH, intense macronutrient deficiency, and are biologically inert. Chaney et al. (2011) showed that a compost made from livestock manure and yard debris supplied N, P, K, and many other nutrients, plus high rates of gypsum ($CaSO_4 \bullet 2H_2O$)

needed to counter the excessive Mg from the $Mg_3Si_2O_5(OH)_4$ mine debris, allowed immediate and persistent revitalization of a site in northern Vermont, USA.

Conclusion

Soils with high levels of phytoavailable potentially toxic elements comprise a harsh environment for plants. Evolution has selected several groups of plants on such soils, the excluders which tolerate metals by not absorbing them, and the hyperaccumulators which accumulate high levels of metals. Fuller understanding of metal phytotoxicity in relation to contaminated sites led to development of *in situ* remediation or phytostabilization of soil metals to protect both plants and wildlife. Soil amendments and agronomic considerations allow effective remediation/revitalization of highly contaminated sites to protect the environment. An alternative phytotechnology, phytoextraction, has been developed to remove metals from contaminated soils. Until government enforced soil remediation generates a market for Cd, As, and other phytoextraction methods, these remain in research. However, Ni phytomining can be profitable, growing hyperaccumulator plants which serve as a high grade ore for Ni. Research continues to further develop the technologies and to help us understand how plants achieve such remarkable reactions to soil metals.

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