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Research Priorities for Conservation of Metallophyte Biodiversity and their Potential for Restoration and Site Remediation

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Abstract

Plants that have evolved to survive on metal-rich soils—metallophytes—have key values that must drive research of their unique properties and ultimately their conservation. The ability of metallophytes to tolerate extreme metal concentrations commends them for revegetation of mines and metal-contaminated sites. Metallophytes can also be exploited in environmental technologies, for example, phytostabilization, phytoremediation, and phytomining. Actions towards conserving metallophyte species are imperative, as metallophytes are increasingly under threat of extinction from mining activity. Although many hundreds of papers describe both the biology and applications of metallophytes, few have investigated the urgent need to conserve these unique species. This paper identifies the current state of metallophyte research, and advocates future research needs for the conservation of metallophyte biodiversity and the sustainable uses of metallophyte species in restoration, rehabilitation, contaminated site remediation, and other nascent phytotechnologies. Six fundamental questions are addressed: (1) Is enough known about the global status of metallophytes to ensure their conservation? (2) Are metallophytes threatened by the activities of the minerals industry, and can their potential for the restoration or rehabilitation of mined and disturbed land be realized? (3) What problems exist in gaining prior informed consent to access metallophyte genetic resources and how can the benefits arising from their uses be equitably shared? (4) What potential do metallophytes offer as a resource base for phytotechnologies? (5) Can genetic modification be used to "design" metallophytes to use in the remediation of contaminated land? (6) Does the prospect of using metallophytes in site remediation and restoration raise ethical issues?

Key words: **conservation of biodiversity, ecosystem, hyperaccumulators, metal tolerance, rehabilitation, remediation engineering, stabilization.**

Introduction

The minerals industry is in transition to environmentally responsible operations, through changes in its economic, environmental, and social practices. Conserving biodiversity is a key component of improved environmental performance, primarily because most mining involves vegetation damage by its clearance or by the surface disposal of wastes, often in pristine areas. One group of plants is especially relevant to conservation efforts by the

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minerals industry—the metallophytes. Metallophytes are species that have evolved biological mechanisms to resist, tolerate, or thrive on the toxic metalliferous soils, and are typically endemic to their native metalliferous soils. Unfortunately, this restricted geographic range is a key factor in their high rates of population decline or extinction, particularly where their mineralized substrates are the target of mining or development. Metallophytes, however, are more than just biological curiosities for conservation; they are the optimal choice for site restoration at mine closure, for the rehabilitation of metal-contaminated land (reclamation and rehabilitation) and the underpinnings for the development of environmental technologies such as phytoextraction of metals from soils (Whiting et al. 2002; see Table 1 for note on definitions).

Metallophytes are the result of tens, hundreds, and often millions of years of strong selective pressures that metal-loaded soils exert on plants (Antonovics et al. 1971; Wild & Bradshaw 1977; Shaw 1990). This results in communities of plants with biological mechanisms enabling them to resist or detoxify the metals within their tissues. The length of exposure to metals governs the degree of specialization of the metal resistance trait. Limited metal resistance is found in some plant populations after a few years of metals entering an environment, for example, around metal smelters. These metal-tolerant races of common plant species (pseudometallophytes) have greater abilities to resist metals compared with members of the same species from clean soils. True metallophytes have evolved on substrates derived from weathered mineral deposits for many thousands or millions of years, and consequently have highly specialized mechanisms of resistance or tolerance. Indeed, metallophytes have often diverged genetically and morphologically to form new taxa endemic to their individual metalliferous area. The majority of these metallophyte taxa are able to tolerate specific metals in the substrate by physiologically restricting the entry of metals into the root and/or their transport to the shoot (termed "excluders" by Baker 1981). A few species, however, have extremely specialized biological mechanisms in that they are able to accumulate, or even "hyperaccumulate" metals in their shoots at concentrations that can exceed 2% of their dry weight (Baker et al. 2000).

Concerted efforts must be made to conserve all metallophyte species, integrating the efforts of scientists, industry, and governments. The minerals industry has recognized the importance of establishing a code of practice for sustainable operation, including consideration of biological diversity. The Mining, Minerals and Sustainable Development (MMSD) project, commissioned by the Global Mining Initiative, was the first industry-wide step towards this goal (available from: http://www.globalmining.com; http://www.icmm.com). The final report of the MMSD was released in May 2002, which, via the 2002 Toronto Declaration, provides a road map for the industry's future contributions to the change to sustainable development (available from: http://www.iied.org/mmsd/). Despite this global focus on conservation of biodiversity

Table 1. A note on definitions.

Ecological restoration, reclamation, rehabilitation and remediation

It is important to be aware of the terminology used to outline the possibilities for use of metallophytes in remedial actions on metal-rich and metal-contaminated substrates. The Society for Ecological Restoration (SER) defines ecological restoration as "the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed" (Society for Ecological Restoration Science and Policy Working Group 2002). In other words, to return a site to its "historic trajectory", rejuvenating the "ecosystem with respect to its health, sustainability, and integrity". The historic conditions are, therefore, an ideal starting point for restoration design. Where the pre-existing conditions of a damaged ecosystem are not comprehensively recorded, the general direction and boundaries of the trajectory can be established by integrating a number of techniques including comparison with an appropriate reference ecosystem.

Under this definition, the term (ecological) restoration can be applied to the use of metallophytes in revegetation only where the site (and/or reference site) originally had those metallophyte species. Unfortunately, as yet, metallophytes are rarely catalogued, and thus the ecosystems at many, if not most, mine sites cannot be ecologically restored.

The majority of uses for metallophytes on metal-contaminated sites will, therefore, be reclamation or rehabilitation, where metallophytes' abilities to tolerate metals are exploited in the establishment of a vegetative cover (not representative of the pre-existing conditions). These processes are not remediation.

Remediation is defined as the process of rendering metalliferous contaminants less toxic, or ideally removing them from the contaminated environment. In terms of using metallophytes for remediation, this will predominantly be the process of phytoremediation (see below).

Phytoextraction, phytoremediation, and phytomining

Phytoextraction is the process of using metal- or hyperaccumulating plants to remove metals and metalloids from soils. The plants sequester the metals in their shoots, which can then be harvested. Two technologies are based on the principle of phytoextraction:

Phytoremediation is the use of metal-accumulating plants to remove pollutants from contaminated soils. This is a transient process because the vegetative cover of metallophytes is removed when the substrate is clean so that the site can be used for other purposes.

Phytomining exploits metal-accumulating plants to recover commercially valuable metals from metal-loaded substrates. Here, the metals are not necessarily considered as contaminants of the soils; the metals may be naturally present at high concentrations because the soils have developed over mineralized bedrock.

sensu lato, the minerals industry and governments at large often do not appreciate the existence and special values of metallophytes. The Robert Brooks Workshop (funded by Rio Tinto Plc. and hosted by the Royal Botanic Gardens, Kew) was one of the first actions directed at conserving and developing the metallophytes resource base (Whiting et al. 2002). Six key questions were debated.

Is Enough Known About the Extent and Global Status of Metallophytes?

Ecosystems on metal-rich soils shelter very diverse biocoenoses, comprised of plants, microbes, and fauna that are specialized to tolerate or avoid the toxic effects of the metals. The scope of ecosystem-level field explorations on metal outcrops has been limited but must be encouraged. Understanding the extent of the metallophytes resource will involve cataloging and conserving the remaining species before any present or future land-clearing activity eliminates them. Three priorities can be identified.

The Need for Field Explorations Using an Ecological Approach

It is essential that geobotanical reconnaissance be initiated on metal-rich soils. Good taxonomic skills are crucial, as are the use of robust ecological techniques. Unfortunately, the number of, and fiscal support for, taxonomists and ecologists is dwindling. There is an urgent need for more precise geobotanical explorations, notably to elucidate the specific links between the plants and their native substrates. Interactions among the different biotic components of the ecosystem must be considered, especially between metallophytes, and the microflora in their rhizosphere. A further necessity is that ecological surveys be conducted before mining activities start. Both the support and motivation to achieve these goals have been, and remain, weak at mining sites. A revolution in mentalities is needed for the inclusion of ecological practice both in exploration and during operation.

Equal efforts must be made to understand and conserve all metallophytes, from pseudometallophyte through hyperaccumulator species. Despite the fact that nonaccumulating metallophytes far outnumber the metal hyperaccumulating species, hyperaccumulator plants attract far more research into their discovery and biological mechanisms. Much of the enthusiasm for finding hyperaccumulator species is driven by the race to commercialize phytoextraction (Baker & Whiting 2002). Additionally, hyperaccumulator species are easier to identify from their phenotype ex situ by chemical analysis of their leaves, or even in situ with colorimetric test kits (Fig. 1) or X-ray fluorescence techniques (Salt et al. 1999). Nonaccumulating metallophytes, on the other hand, can only be recognized as metal resistant by a tacit assumption based on their existence on metal-rich substrates, or by rigorous tolerance testing in the laboratory. Consequently, the exact number of metal-resistant species is not known, but there may be hundreds of thousands of species. The extent of metal hyperaccumulating taxa is better known, for Ni (about 330 species), Zn (12), Mn (10), Co (30), Cu (32), Se (20), Pb (14), As (5), and Cd (2), but these numbers are certainly set to increase (Reeves 1992; Reeves & Baker 2000).

Figure 1. Field testing for high Ni concentrations in plant leaves using a colorimetric reagent, dimethylglyoxime. The plant, *Phyllanthus balgooyi*, is a Ni-hyperaccumulator endemic to ultramafic soils in Palawan and Sabah (Philippines).

Focus on Metallophyte Hotspots—and Elsewhere!

Surveying should focus on "hotspots" with high diversity and endemism of metallophytes, including natural outcrops and also mine, smelter, and other industrial wastes rich in metals. Several regional heavy metal outcrops may be recognized as major foci of metallophyte diversity: Latin America, Southeast Asia, Southern Africa, China, Mediterranean Europe, Cuba, and New Caledonia. The south-central African copper outcrops provide a good example, of which about 550 metallophyte taxa have been identified to date (Brooks & Malaisse 1985). At least 40 of these taxa are habitat specialists (holoendemics) confined to this small area, even to one or two isolated locations, while more than 30 species can hyperaccumulate Cu and/or Co. In the same Zambezian region, serpentine outcrops of the Zimbabwean Great Dyke have yielded 350 metallophytes, 26 endemic taxa, and five Ni hyperaccumulators.

A strong focus on the flora of serpentine and lateritic soils exists because ultramafics have the greatest extent of all metalliferous soils, and have also given rise to the most extensive evolution of new species, which often remain endemic to regions of this soil type (Baker et al. 1992). The restriction of many species to localized ultramafic soils means that many metallophytes, whether hyperaccumulators or not, are very rare. Recent fieldwork on serpentinophytes in Brazil (Brooks et al. 1992), Cuba (Reeves et al. 1999), South Africa (Morrey et al. 1992), Australia (Batianoff et al. 1991, 2000), and Turkey (Reeves et al. 2001) has yielded new species to science, which include further instances of hyperaccumulation. Many ultramafic floras, especially, in the tropics, remain inadequately investigated. Reeves' (2003) analysis of herbarium specimens discovered further cases of Ni hyperaccumulation by plants from Puerto Rico, Australia, Indonesia (Sulawesi), Japen, and Sabah. The incomplete identification of three of these tropical Ni hyperaccumulators is symptomatic of the need for greater exploration of the world's metallophyte-hotspots and more detailed taxonomy. Other tropical ultramafic floras needing study include those of Guatemala, Costa Rica and Venezuela, parts of Brazil beyond Goiás state, New Guinea, islands of Indonesia (e.g., Waigeo and Gebe), and many of the islands of the Philippines (Luzon, Mindanao, Palawan, Samar, Bicol, Nonoc, and Dinagat).

Although these hotspots deserve detailed investigation, other metallophytes must not be forgotten. There are countless areas or regions of metal-rich soils around the globe, whether natural or anthropogenic, that cannot be considered "hotspots", but should not be neglected from study. Indeed, much, if not most, of the influential research work on metallophytes to date has been on metal-tolerant plants from these areas. This work must continue alongside the study of plants in hotspot areas. For example, there are many small areas of metal-rich soils supporting metallophyte species that can be found in a number of recognized habitats (e.g., in Annex 1 of the European Habitats Directive 1992 92/43/EEC).

The Need to Develop the Resource Base: Databases, Germplasm, and Living Collections

Concerted efforts must be directed at protecting the native environments of metallophytes. However, this will often conflict with operations to extract the mineral wealth from these areas. A critical requirement for conserving metallophytes is, therefore, assembling collections before mining activities, according to (standardized) methodologies that account for (i) the diversity within the ecosystem; (ii) genetic diversity within each species/population, for example, by maintaining "core collections" (Diwan et al. 1995); and (iii) species that have sustainable uses in ecological restoration and other phytotechnologies. It is, therefore, urgent to establish metallophyte collections. These can either be in situ, for example, by leaving representative biotope "islands", or ex situ, for example, in seed gardens, arboreta, botanic gardens, or germplasm banks. Databases of metallophyte species and of research are also crucial.

Collections of seed or germplasm of metallophytes are vital for basic research, conservation of genetic resources, selection, and large-scale breeding activities. The danger of a valuable natural resource becoming extinct before its properties and distribution are properly known is ever present. There is no germplasm facility dedicated to metallophytes although in the last decade a number of small seed collections of metallophytes have been established as "banks". The Universities of Melbourne and Oxford now manage a seed bank of (largely European) metal hyperaccumulator plants. The Australian Center for Mining Environmental Research (available from: http://www.acmer.com.au) has also supported work on the collection and use of native seed for the revegetation of Australian mineral wastes, as have nationally funded programs (e.g., FloraBank and Greening Australia). Initiatives such as the Kew Millennium Seed Bank project might aid preservation of metallophytes. To underpin this activity, however, there is an urgent need for a fuller understanding of storage and viability requirements for seed of metallophytes.

Geobotanical survey data from mineralized areas must be collated in accessible databases. Attempts to produce such databases have been few and global coverage is patchy. The most extensive are Environment Canada's PHYTOREM database which currently supports data on about 800 metallophytes, and the METALS (metal-accumulating plants) database maintained by Environmental Consultancy, University of Sheffield (ECUS Ltd, U.K.) A great benefit would be the integration of such databases on a global scale, and their availability through the World Wide Web. These databases must assimilate research into life cycles, associated species (microbes, plants, etc.), propagation, resistance to disease and predation, etc. Often nothing is known beyond the morphology and location given in the publication where the species was first described. This is clearly inadequate for judging the potential of a species for further cultivation and uses.

Integrated global efforts are, therefore, required to conserve metallophytes. An unknown number of metallophytes will have already become extinct. Governmental policies and international treaties such as the Convention on Biological Diversity (CBD) that encourage the identification and conservation of metallophyte biodiversity will help protect those species that remain, as will the discovery of uses for their unique properties. At the company/stakeholder level, metallophyte conservation might be incorporated in Environmental Management Systems as guided by ISO 14000.

Are Metallophytes Threatened by the Activities of the Minerals Industry, and can their Potential for the Restoration or Rehabilitation of Mined and Disturbed Land be Realized?

Mining is probably responsible for the destruction of the majority of metallophyte habitats and the associated loss of species. The most direct mechanism for ensuring the survival of metallophytes in mined areas is to promote their use in ecological restoration and site rehabilitation at mine closure. The adoption of sustainable development policies necessitates that land rehabilitation considerations be incorporated into mine planning by mining companies such that it becomes a major governing factor in the initiation and management of mining operations, waste disposal, and site closure (Johnson et al. 1994). From an environmental perspective, mining causes the destruction of natural ecosystems through removal of soil and vegetation and also their burial beneath waste disposal sites. Thus the ecological and sustainable approach to the rehabilitation of mined land in practice can largely be considered as ecosystem restoration—the reestablishment of the land's ecological integrity, its structure, function, and biodiversity (Cooke & Johnson 2002).

Environmental best practice in mining can and should incorporate the goals of both the conservation of metallophytes for future generations, their use in site rehabilitation and the exploitation of their unique genetic properties in environmental technology. The use of metallophytes in ecological restoration sensu stricto (Table 1) is likely to be small in comparison with the huge potential they have for site rehabilitation and remediation.

To date, metallophytes have had a particular place within revegetation strategies for metalliferous sites, and the practical use of metal-tolerant plant populations (mostly grasses) to stabilize and revegetate waste is well known. In particular, the use of ecotypes of the temperate grasses *Agrostis capillaris* L. (common bent-grass) and *Festuca rubra* L. (red fescue) (both Gramineae) is a proven rehabilitation technology of over 20 years' standing for dealing with medium toxicity Pb, Zn, and Cu mine tailings (Smith & Bradshaw 1979; Johnson et al. 1994). These ecotypes have metal tolerance as a genetically heritable character, and some have been bred on to cultivar status (e.g., *F. rubra* cv. "Merlin"). These metal-tolerant plants can be combined with a minimal covering of a suitable substrate to stabilize the metalliferous wastes. For example, in 1977 the revegetation at Parc Pb/Zn mine in North Wales used 100 mm of quarry shale seeded with a mixture of tolerant *F. rubra* cv. "Merlin" and the legume *Trifolium repens* L. (white clover) (Leguminosae), which can naturally increase the nitrogen content of the substrate. The tolerant fescue provided a bioengineering solution to the erosion problems by rooting well through the shale cover and into the tailings, thus giving the site physical stability. This vegetation cover persists to the present day.

The widespread use of local species native to the metal-contaminated area to be revegetated has been restricted (Tordoff et al. 2000). For true ecological restoration of sites degraded by mining and processing activities, the template developed by the Science and Policy Working Group of the Society for Ecological Restoration should be applied (Society for Ecological Restoration Science and Policy Working Group 2002). There are a number of key biological problems concerning metallophytes and their role in ecological restoration of mine sites that also need research.

(1) Identifying and understanding metal-tolerance in local metallophytes native to the specific mining area;
(2) Encouraging the commercial production of suitable native species and their seeds;
(3) Overcoming slow grow rates typical of stress-tolerant species and improving sward ground cover in metal-tolerant grasses;
(4) Reducing fertilizer inputs and identifying nitrogen-fixing metallophytes to promote "low-maintenance" vegetative cover;
(5) Developing metallophytes with multiple metal-tolerance systems for use on heterogeneous wastes and other chemically complex mining substrates;
(6) Developing metal-excluding plants to minimize transfer of metals into the food chain on the restored sites (both livestock and native fauna); and
(7) Post-revegetation chemical and ecological monitoring of restored sites to provide case studies of longer-term ecosystem development.

The minerals industry should understand the importance, potential value, and methods of conservation of local indigenous metallophytes as key species in the restoration or rehabilitation of metalliferous mineral deposits, perhaps by incorporating these concepts as an integral part of owning mineral rights. The recent history of mining in ultramafic areas of New Caledonia illustrates a number of important points. New Caledonia has a very large endemic flora restricted to ultramafic soils and many, if not most, of the 1130 plant species are metallophytes. Yet the past mining practices have not only largely ignored the importance for global conservation of this hotspot of metallophyte diversity but also the potential use of these indigenous plants in restoring the 120 years legacy of mining—over 200 km^2 of severely degraded mined land. In fact, local metallophytes such as nitrogen-fixing trees (*Gymnostoma* spp., Casuarinaceae) were ignored until the 1980s (Fig. 2). Clearly the aim in New Caledonia, and globally, should be no less than the restoration of the unique natural vegetation (Bradshaw 1997; Tordoff et al. 2000; Society for Ecological Restoration Science and Policy Working Group 2002). Such best practices will help to maintain the future of mining within the context of sustainable development as more mining projects occur in remote wilderness areas and fragile ecosystems, where innovative

Figure 2. Restoration trial in New Caledonia with endemic metallophytes including *Gymnostoma leucodon* (Casuarinaceae) and *Schoenus juvenis* (Cyperaceae).

and creative ecological restoration methods will be needed.

What Problems Exist in Gaining Prior Informed Consent to Access Metallophyte Genetic Resources and how can the Benefits Arising from their Uses be Equitably Shared?

A template for the conservation and sustainable uses of metallophytes is provided by the CBD, incepted at the 1992 Earth Summit in Rio de Janeiro (available from: http://www.biodiv.org). The CBD entered into force in December 1993 and has been ratified by 179 countries. The CBD is an international treaty and thus a source of international law. The core objectives of the CBD are the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of benefits arising out of the use of genetic resources.

The CBD has several implications for both the study of metallophytes and for industrial activity in metal-rich areas (not only metallophytes in their natural habitats, but also those on reworked metalliferous surfaces from past mining activity as well as plants found on tailings, settling tanks and wastes, and also in ex situ collections). For example, the CBD calls on Parties to identify and monitor biodiversity, identify adverse processes and manage their biological resources; it requires Parties to adopt measures relating to the use of biological resources to avoid or minimize adverse impacts on biological diversity. It is relevant to the ex situ commercial applications of metallophytes because concerns surrounding alien species have been identified as an issue cutting across many articles and thematic programs of the CBD.

Access to Metallophytes and Benefit Sharing

Countries have sovereign control over access to their genetic resources (natural capital), but are obliged to facilitate access. However, those seeking access must obtain the prior informed consent of the country, by telling those responsible for access to genetic resources what they want, what they are going to do with it, and get their consent to proceed. They must also negotiate mutually agreed terms for access to results, benefits sharing (e.g., royalties and technology transfer) and, where possible, carry out joint research with the provider country. This access and sharing has several implications that may be relevant to metallophyte research. Given national sovereignty, the CBD recognizes the host country's authority to determine access to genetic resources and some 50 countries have now developed or are developing access laws. These measures govern access to genetic resources, biochemical compounds and traditional knowledge by companies and other collectors. In terms of economics, the markets based on products derived from genetic resources are an estimated 500–800 billion US$ (ten Kate & Laird 1999). There are arguments about how much is derived from genetic resources per se and how much by technological developments based on them. The point is that substantial sums are involved and this is a key factor in the decision to draw up access laws.

Problems and Practicalities

A number of issues mitigate against easy access to metallophytes for research, and against the transportation, distribution, or sharing of metallophyte germplasm necessary to pursue research and development. It is not always clear who should be contacted in the provider country to legally gain access to metallophytes, for prior informed consent and negotiation of benefit sharing. Some countries have allocated rights to exploit mining sites to individuals, military officials, or multinational companies. Permission from indigenous people or local communities from surrounding areas may also be required. On the other hand, there is a perception within the scientific and research community that genetic resources are the property of no one. This clearly contravenes the CBD.

There are increasing pressures to employ, enhance, or genetically modify metallophytes for use in remediation, but there is a lack of regulatory oversight, which is important because these plants may be endangered, not commercially available, or they may be invasive in a non-native habitat. This is compounded by a lack of scientifically validated techniques to evaluate sustainability of biodiversity-based processes or products. There are also problems associated with regulation, transport, and storage in the transfer of candidate cultivars for phytotechnology that could be considered as contaminated materials or dangerous goods in some jurisdictions.

Action Required

All these factors are obstacles to the use of metallophytes in research. How can a course be steered which facilitates access to metallophytes, ensures the fair and equitable sharing of any resultant benefits and ensures that researchers act legally? The first step is to clarify the value of metallophytes to science, industry, governments, and broader society, perhaps by the establishment of a Metallophytes Working Party. There are two reasons for this. Conservation of metallophytes in their indigenous habitat will only be assured if interested stakeholders broadly understand their value. Second, it is important to outline the value of metallophytes so that all parties have a clear idea of what benefits might accrue from their study. This will make negotiation of benefit sharing much easier. Benefits do not need to be financial.

The CBD calls for provider countries to identify a focal point for issues surrounding access and benefit sharing. In countries where the process of gaining legal access is unclear, the mining industry could help encourage governments to identify such a focal point, to facilitate access to metallophyte genetic resources. Many companies and researchers may perceive access laws to be restrictive and a barrier to science. However, it is too easy to blame the country of origin if user communities have not been proactive in helping to create a framework which allows access via prior informed consent and benefit sharing. It is in the interests of companies and scientific institutions to work with countries in developing access and benefit-sharing agreements. It might, therefore, be advantageous to concentrate research on metallophytes from one or two countries initially. A coordinated approach from researchers, using case studies to illustrate the value of metallophytes, the benefits from research, and how they can be shared with the provider country, could lead to an access and benefit-sharing agreement. This would prevent each individual worker having to contact and negotiate agreements with provider countries independently. Such an agreement could then be used as a template for further research in other countries.

What Potentials do Metallophytes Offer as a Resource Base for Phytotechnologies?

Metallophytes offer huge potential for the development of environmental phytotechnologies. Metallophytes can be used for rehabilitation and revegetation of degraded metal-polluted areas. They can also be used to remediate contaminated land by exploitating their metal-accumulating properties to scavenge metals from metalliferous soils (phytoextraction). There are many thousands of species of metal-tolerant, nonaccumulating plants that might be considered for phytostabilization. These species are unified by the fact that they restrict the transfer of metals to their shoots, which will reduce the entry of metals into the food chain. Conversely, for phytoextraction, fewer than 500 species have, to date, been found to have the ability to hyperaccumulate metals at concentrations which are between 10^2 and 10^5 times greater than in "normal" plants (Baker 1981; Baker et al. 2000).

Revegetation

Establishing vegetative cover is one of the best ways to prevent metal migration from metal-contaminated sites via erosion, and metal-rich dusts or leachate. The long-term stability that the plants provide in terms of preventing metals from leaving the site means that this technology is often termed phytostabilization. Phytostabilization has widespread application for capping sites contaminated with metals. Phytostabilization is used to vegetate metal-contaminated waste sites that tend to be recent, and consequently do not have naturally adapted ecosystems of metallophytes, including highly engineered facilities such as decommissioned tailings dams.

Metal-tolerant plants that do not accumulate metals in their shoots are selected for phytostabilization to minimize metals entering the food chain. The value of establishing a persistent vegetative cover for preventing pollutant movement has long been known. One strategy is to establish metal-tolerant vegetation directly on the metalliferous substrate. An alternative is to modify the substrate physically and chemically to render it less toxic, permitting the growth of plants with limited metal tolerance. A push to understand the chemical dynamics of metals in soils has yielded a highly promising phytostabilization technology. This technology uses "tailor-made" amendments of P- and Fe-rich organic wastes (biosolids or manure/compost) plus alkaline amendments, coupled with metal-tolerant excluder plant species. The technology has been used with demonstrated successes on Pb-, Zn- and Cd-contaminated sites in the U.S.A. and Poland, and on Ni-contaminated sites in Canada (Daniels et al. 1998; Brown & Chaney 2000; Li et al. 2000; Kukier & Chaney 2001; Brown et al. 2003a,2003b). Considerable research efforts are needed to continue screening vegetation for its ability to tolerate metals by excluding them from the aerial parts, and to continue to improve the efficacy of amendments.

Phytoextraction

Phytoextraction has the most promise for revenue generation, but the technology is still in its infancy (Brooks 1998; van der Lelie et al. 2001). Only a few of the hyperaccumulator species may be commercially viable in phytoextraction. The feasibility of phytoremediation depends on both the level of contamination in the soil and the mass of metal that can be extracted by each crop. For many metal-rich wastes it would be very difficult or even impossible to clean up these sites with phytoremediation (Zhao et al. 2003). Stabilization using metal-tolerant plants is then a more logical choice. On some sites, phytomining to extract Ni, Co, Tl, and Au for their economic value may be possible, generating a metal-rich smelter feedstock ("bio-ore"); the driving factor here is the high dollar-value of these metals (Brooks et al. 1998; Anderson et al. 1999). Cleaning up metal-contaminated soils by phytoremediation is most feasible on low to moderately contaminated soils such as agricultural land impacted by the application of low-level metal sources, for example, sewage sludge or atmospheric deposition. Here, the burden of metals might be extracted by phytoremediation in as few as 3–5 years (Zhao et al. 2003).

Key research priorities for phytoextraction are:

(1) Scientific understanding of the physiological, molecular, and genetic mechanisms of metal hyperaccumulating metallophytes;
(2) Screening and breeding hyperaccumulating plants for higher biomass and/or higher metal accumulation;
(3) Development of agronomic practices, for example, planting and harvest dates, methods for planting and harvest, plant density : yield trade-offs, nutrient additions, light, water, temperature, soil conditions, plant protection, weed and pest control, and determination of annual versus perennial (regrowth) management (Chaney et al. 2000);
(4) Methods for processing the biomass, including incineration, metal extraction from the biomass or its ash, and disposal in landfills. Notably, the energy produced during ashing can be harnessed and could change a metal phytoextraction model enough to be very economical in developing countries; and
(5) Environmental risk assessment of phytoextraction crops, for example, their impact on the food chain.

Between phytoextraction and phytostabilization, sites contaminated by many metals can be effectively remediated to prevent erosion and initiate a healthy ecosystem. Inexpensive by-product amendments can also be used to improve soil fertility to support phytostabilization and phytoextraction. The low cost of these methods compared with soil removal and replacement ("dig and dump") could provide great public benefit. Given sufficient funding and working field demonstrations, these phytotechnologies have the potential to become billion dollar industries.

Can Genetic Modification be Used to Design Metallophytes for Use in the Remediation of Contaminated Land?

The use of metallophytes in phytostabilization, phytoremediation, and phytomining is recognized as an environmentally desirable goal, but there are a number of hurdles to be overcome before this objective can be realized in practice. One of these is the rarity of most metallophytes, many of which have very limited population sizes that are threatened by industrial development (Whiting et al. 2002, 2003). Therefore, nonindigenous or engineered metal-tolerant plants will need to be considered. Extensive research is being directed at the selection of genotypes or cultivars with favorable growth characteristics (such as metal-tolerant high-biomass brassicas, Salt et al. 1998), or genetically modified (GM) plants that can tolerate and hyperaccumulate specific metals (Pilon-Smits & Pilon 2002). Successful development of improved metallophyte crops will require a detailed understanding of the underlying biological mechanisms.

Dissecting the traits that characterize metallophytes that have naturally evolved on metal-rich soils is the obvious starting point for "designer" metallophytes. The physiological and genetic mechanisms of metal tolerance in non-accumulating metal-tolerant metallophytes is becoming well elucidated (Karenlämpi et al. 2000; Clemens 2001; Hall 2002), and appears, in certain cases, to be under the control of a relatively few genes (e.g., Schat & Vooijs 1997; Smith & Macnair 1998). A better understanding of the processes of metal tolerance and homeostasis will ultimately be important as a basis for more targeted strategies for developing metal-tolerant crop plants and plants for phytostabilization.

The biochemical and genetic basis of metal-hyperaccumulating metallophytes, on the other hand, is far from being revealed (Pilon-Smits & Pilon 2002; Pollard et al. 2002). Hyperaccumulator plants have a more complex genetic background because of the many mechanisms of metal transport, homeostasis, binding, and sequestration. By analyzing each process individually, a model of how these species function is gradually being built. Rapid advances in biophysical and chemical analytical techniques are providing key tools for characterizing the complexation of the metals in situ in plant cells. Similarly, the explosion in the number of molecular biological tools available for dissecting genetic mechanisms is revealing a long list of genes available to improve plants for phytoextraction and phytostabilization in the next decade.

The most widely studied "model" Zn hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri* (both Brassicaceae) exhibit broad inter- and intrapopulation variation in metal accumulation and tolerance, providing good opportunities for formal genetic analysis and the molecular cloning of candidate genes (Pollard et al. 2002). Given the number of research groups focused on these model species, a full understanding of hyperaccumulation may not be a too distant prospect. This must include all of the key processes from the ground up if effective transgenic hyperaccumulators are to be developed for phytoextraction. The exceptional concentrations of metals in the shoots of hyperaccumulator plants demonstrate the linkage of effective metal-acquisition mechanisms by the roots with a high degree of cellular tolerance to metals within the plant. The sequencing of complete plant genomes and development of new tools in bioinformatics and functional genomics will greatly facilitate gene discovery.

Major steps for complete biochemical and genetic elucidation include:

(1) *Bioavailability and acquisition:* Production of metal-mobilizing compounds by the roots (e.g., McGrath et al. 2001; Takahashi et al. 2001); root size, architecture and metallophilic foraging traits (Whiting et al. 2000); the role of root-associated microbes (Kamnev & van der Lelie 2000; Whiting et al. 2001).
(2) *Metal trafficking and homeostasis:* Understanding the critical roles of ion-transport across the plasma membrane of cells (Pence et al. 2000; Assunção et al. 2001; Persans et al. 2001; Lombi et al. 2002; White et al. 2002).
(3) *Detoxification:* The production of metal-binding ligands to detoxify and facilitate transport and storage of metals (Krämer et al. 1996; Salt et al. 1999; Meagher 2000; Cobbett & Goldsbrough 2002).
(4) *Sequestration:* The mechanisms controlling the biochemical processes in metal compartmentation at the organ and cellular level (Küpper et al. 1999, 2001).

Does the Prospect of Using Metallophytes in Site Remediation and Reclamation Raise Ethical Issues?

Little is known about the risks that might be associated with using metallophyte plants for site remediation ex situ from their native environments. There may be public concern over the use of a nonindigenous fast-growing crop that takes up extreme concentrations of toxic elements. This concern, whether real or imagined, is likely to be magnified if that crop has been GM (Linacre et al. 2003). Perhaps the most significant concern with either of these scenarios is simply the potential for metal-accumulating plants to escape from the site of cultivation and become established as a new weed within ecosystems.

The primary mechanisms for the unintended spread of released species into ecosystems are via the dispersal of seed. Seeds of many hyperaccumulators are small and can be carried by the wind. The fact that many wild hyperaccumulator plants have remained endemic to metalliferous soils suggests that spread by this route could be unlikely; hyperaccumulator plants appear less able to survive or establish viable populations on low metal soil, thus posing a reduced hazard for establishment beyond the initial area of planting. This is perhaps because the high metal content of the tissues confers protection against root and shoot pathogens and herbivores (Pollard et al. 2002). Further research must establish the competitive ability of non-native metallophyte species to be used for remediation projects, and their ability to establish in low metal environments.

A number of other potential risks must be considered to enable the acceptance of technologies based on metallophytes or their derivatives. Pollen of natural or GM metallophytes might move via wind or insects with potential for gene flow or introgression into wild and agronomic relatives. An additional consideration for using metallophytes is the potential for transfer of metals up the food chain if metals are assimilated into the plant tissue. These potential risks have received scant attention, with few studies targeted directly at assessing the scale of the threats, if any, posed (Pilon-Smits & Pilon 2002; Wolfe & Bjornstad 2002; Rock 2003). Any risks must be interpreted in the context of the permanent risk posed by leaving contaminated sites untreated, which may represent a more direct threat to human health. This raises an interesting legislative hurdle, which might deter the use of metallophytes for rehabilitation in the U.S.A., and probably many of the developed countries. Mining and environmental quality laws require that any metalliferous soils left behind must be covered with uncontaminated substrate to avoid the introduction of metals to the food chain, thus obviating the need to develop metallophytes for rehabilitation. This is unfortunate, because there are many abandoned hectares of metal-contaminated lands for which there is no funding for cap material, and no revegetation prescription. Consequently, there is a real need to develop integrated risk assessment, management, and communication strategies for metallophyte-based technologies to be acceptable to the public and to regulators (Linacre et al. 2003).

Conclusions: Securing a Future for Metallophytes

Metallophytes are widespread throughout the world, forming an integral part of the biodiversity on metalliferous soils. The island-like nature of metalliferous outcrops has assisted the evolution of many endemic metallophyte species, which make an important and disproportionately large contribution to global biological diversity. The native vegetation of these metalliferous areas can easily be lost in the early stages of mine site development, and the restricted distribution of these species leads to absolute rather than local extinctions. Concerted efforts must, therefore, be directed at cataloguing and conserving metallophytes from the onset of operations at any site, with structured methodology for conserving them both in and ex situ. Promoting metallophytes awareness and recognition that they have commercial uses is undoubtedly the vital first stage.

The bottom line is that the extent of our understanding of the biodiversity, biological mechanisms and biotechnological applications for metallophytes is fragmentary. There are certainly tens of thousands more species of metallophytes that remain to be discovered, of which maybe a few hundred will hyperaccumulate metals. The issues identified here should be the primary foci for future research and efforts to conserve metallophytes. Such research should be strongly supported by the minerals industry, which in many cases is ideally placed both to ensure the conservation of metallophytes and to exploit their unusual properties. Systematic screening of plants on metalliferous sites, particularly those likely to be the focus of future mining, will identify priority candidates for conservation, for implementing ecological restoration of mine sites, and for the development of "green" technologies for removing metals from the soil.

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