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PHYTOREMEDIATION: AN AFFORDABLE GREEN TECHNOLOGY FOR THE CLEAN-UP OF METAL-CONTAMINATED SITES IN SRI LANKA

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ABSTRACT

Phytoremediation, the use of plants to remediate sites contaminated with organic and inorganic pollutants, and phytomining, the technology involved in extracting the pollutants removed for commercial purposes, are rapidly-growing industries with multi-million dollar markets. This solar-driven, green technology is often favored over more conventional methods of clean-up due to its low cost, low impact, and wider public acceptance. In this paper we discuss phytoremediation as a valid alternative for remediating contaminated bodies of soils and water in developing countries like Sri Lanka, where clean-up can often be stalled due to the high costs associated with traditional remediation technologies. While phytoremediation techniques clearly have limitations, a carefully-planned phytoremediation-phytomining operation using native, fast-growing, deep-rooted, high-biomass species, will not only remediate contaminated sites, greatly reducing exposure to contaminants by humans and wildlife, but also generate income from otherwise barren land unsuitable for agricultural or recreational purposes.

INTRODUCTION

Large areas of the world have been contaminated with organic and inorganic pollutants. Organic pollutants are mostly anthropogenic in origin and are released into the environment via solvent and fuel spills, military operations, agricultural practices, and industrial activities. Organic pollutants can include solvents such as trichloroethylene (TCE), a common ground-water pollutant (Newman *et al.*, 1997), herbicides such as atrazine (Burken and Schnoor, 1997), explosives such as trinitrotoluene (TNT) (Hughes *et al.*, 1997), hydrocarbons such as oil, gasoline, benzene, toluene, and polycyclic aromatic hydrocarbons (PAHs) (Schnoor *et al.*, 1995, Aprill and Sims, 1990), fuel additives such as methyl tertiary butyl-ether (MTBE) (Hong *et al.*, 2001), and the much-discussed polychlorinated biphenyls (PCBs) (Harms *et al.*, 2003). Inorganic pollutants, on the other hand, occur as natural elements in the Earth's crust. Inorganic pollutants can be plant macronutrients such as nitrates and phosphates, micronutrients such as Cr, Cu, Fe, Mn, Mo, Ni and Zn, nonessential elements such as As, Cd, Co, F, Hg, Se, Pb, V, and W, and radionuclides such as ^{238}U, ^{137}Cs, and ^{90}Sr (Dushenkov, 2003). Human actions such as mining and smelting, industry, traffic, agriculture, waste disposal, and military activities often release these inorganic pollutants in high - often toxic - concentrations. Mining, for example, can significantly increase the rate of heavy metal release into the environment, both onto the soil surface and into ground water. The most adverse effects of mining and other anthropogenic activities are often felt after the activity has been discontinued (Banks *et al.*, 1997; Petrisor *et al.*, 2004). Pollutants, whether organic or inorganic, severely impact human health, productivity of agricultural lands, and the stability of natural ecosystems (Bridge, 2004). Widespread contamination of agricultural lands, for example, has significantly decreased the extent of arable land available for cultivation worldwide (Grêman *et al.*, 2003). Many of these pollutants are also known carcinogens.

Heavy metal contamination has increased dramatically since the early 20th century (Nriagu,

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1979; Ensley, 2000). Unlike some organic pollutants, most inorganic pollutants, such as heavy metals and radionuclides, cannot be eliminated by chemical or biological transformation. Although it is possible to reduce their toxicity by influencing chemical speciation, heavy metals do not degrade and are generally persistent in the environment (Cunningham and Ow, 1996). The costs associated with the clean-up of organic and inorganic pollutants can be staggering, even for developed countries. Currently, US $ 6-8 billion is spent annually for environmental cleanup in the United States alone, and US $ 25-50 billion is spent per year worldwide (Tsao, 2003). Recent estimates suggest that to effectively clean-up 1200 of the United States' most contaminated and abandoned sites, the so-called Superfund sites, an estimated US $ 700 billion would be required (Glass, 1999, 2000). The costs involved in remediation of more than 33,000 contaminated sites in Europe are equally overwhelming (Adriano, 2001).

The various remediation technologies currently used range from *in situ* vitrification and soil incineration to excavation and land filling, soil washing, soil flushing, and solidification and stabilization by electrokinetic systems (Glass, 1999). These engineering-based technologies are most appropriate for highly polluted sites and are often not suited for the treatment of widespread yet low levels of contamination found in many parts of the world. Conventional methods also contribute to further environmental degradation and are prohibitively expensive when a large area of land or water is involved (Ensley, 2000). The costs involved in engineering-based technologies for developing nations such as Sri Lanka are unbearably expensive. This is an especially important consideration for Sri Lanka and regions in South Asia where heavy metal and organic pollutant contamination already pose a severe threat to human and ecosystem health (WHO, 2003). Although there are only a few published reports examining the nature of soil and water contamination in Sri Lanka (Allinson *et al*., 2002; Bandara, 2003), it is likely that large areas may contain high levels of heavy metals and other pollutants due to discharge of often unregulated and untreated factory effluents (Dissanayake *et al.*, 2002). The degraded quality of the water resources, for example, is a major environmental concern in Sri Lanka, with agrochemicals, untreated industrial effluents, domestic waste water, and the unregulated disposal of solid waste including hazardous material, all contributing to the contamination of both surface and ground water resources (Bandara, 2003).

Given the nature and extent of contamination worldwide and the costs involved in remediation, recent years have seen a drive toward alternative yet effective technologies for the remediation of polluted sites. In this regard, bioremediation, typically referring to microbe-based clean-up, and phytoremediation, or plant-based clean-up, have generated much interest as effective low-cost and environmentally-friendly technologies for the clean-up of a broad spectrum of hazardous organic and inorganic pollutants (Pilon-Smits, 2005). Plant-based environmental remediation has been widely pursued by academic and industrial scientists as a favorable low-impact clean-up technology applicable in both developed and developing nations (Raskin and Ensley, 2000; Robinson *et al.*, 2003a, b). Companies specializing in phyto-remediation have emerged in many developed and some developing nations to service a growing global market; the US market alone is estimated to be about US $150 million per year (Glass, 1999, 2000). Given the low-cost and widely effective nature of phytoremediation, it is likely that this green technology is the only alternative for developing nations, such as Sri Lanka, where clean-up is hindered by a lack of funding. Phytoremediation can also be an income-generating technology, especially if metals removed from the soil can be used as bio-ore to extract useable metal, *i.e*., phytomining (Brooks *et al.*, 1998; Angle *et al.*, 2001), and energy can be generated through biomass burning (Li *et al*., 2003). Phytomining is now a fast-developing field with the potential to generate income by exploiting low-grade ore bodies that are not economical to mine by conventional methods. The overall outcome of a carefully-planned phytoremediation-phytomining operation would be a commercially viable metal product (*i.e*., metal-enriched bio-ore) and land better suited for agricultural operations or general habitation (Boominathan *et al.,* 2004). Substantial research efforts are currently underway to realize the economic potential of these green technologies (Ghosh and Singh, 2005) with several plant species now recognized as suited for the phytoremediation-phytomining of Ni, Co, Tl, Pb, Cu, Zn (Anderson *et al.*, 1999; Chaney *et al.*, 1997; Brooks *et al.*, 2001; Boominathan *et al.,* 2004), radionuclides

(Dushenkov, 2003), and even As and Au (Anderson *et al.*, 1998; Mohan, 2005; Meharg, 2002; Anderson *et al*., 2005; Visoottiviseth *et al*., 2002).

PLANTS FOR PHYTOREMEDIATION

The success of phytoremediation or phytomining depends on the availability of plant species - ideally those native to the region of interest - able to tolerate and accumulate high concentrations of heavy metals (Baker and Whiting, 2002). Some plants can accumulate remarkable levels of heavy metals: 100-1000-fold the levels normally found in most species. This striking phenomenon, known as metal hyperaccumulation (*i.e*., the ability to accumulate at least 0.1% of the leaf dry weight in a heavy metal), is only exhibited by < 0.2% of angiosperms (Baker and Whiting, 2002), making the selection of native species for phytoremediation efforts a difficult task. Many hyperaccumulating species, characterized by their tolerance to toxic levels of metals such as As, Co, Cu, Zn, Mn, Pb, Se, Ni, and Cd, are often endemic to metal-rich substrates and are rare in their distribution (Baker *et al*., 2000). Currently there are about 420 species belonging to about 45 plant families recorded as hyperaccumulators of heavy metals (Cobbett, 2003). While new hyperaccumulators continue to be discovered from field collections (Krämer, 2003) only a few species have been tested in the laboratory to confirm their hyperaccumulating behaviors. The rush to discover hyperaccumulators (Ernst, 2000; Baker and Whiting, 2002), however, has so far shown several intriguing patterns. First, several plant families contain a inexplicably high number of hyperaccumulators: among those are Asteraceae, Brassicaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, and Violaceae, suggesting that several families and genera within them may be preadapted/predisposed to deal with high concentrations of metal. Secondly, there appears to be a disproportionately high percentage of hyperaccumulators in tropical regions. For example, two-thirds of the 320 species of Ni hyperaccumulators so far discovered are found exclusively in tropical regions (Reeves, 2003; Proctor, 2003). Thirdly, over 80% of the known metal hyperaccumulators accumulate Ni rather than other metals. The reasons for these intriguing patterns likely result from a combination of factors including: 1) more time available for the evolution of these species in tropical regions, 2) greater weathering of naturally occurring ore-bodies in tropical regions, imposing stronger selective pressures on local floras, 3) traits conferring metal hyperaccumulation providing a selective advantage in dealing with other stresses of tropical habitats (*i.e.*, drought, disease, herbivory), 4) greater availability of Ni over other metals due to the presence of vast tracts of Ni-enriched serpentine outcrops along continental margins, or 5) perhaps a greater interest among botanists for the search for hyperaccumulators in tropical regions.

New Caledonia is a biological hotspot for Ni hyperaccumulators with over 50 species found endemic to the metal-rich serpentine outcrops of the island (Reeves, 2003). These include species from genera such as *Argophyllum* (Grossulariaceae), *Casearia* (Flacourtiaceae), *Cledion* (Euphorbiaceae), *Geissois* (Cunoniaceae), *Homalium* (Flacourtiaceae), *Hybanthus* (Violaceae), *Oncotheca* (Oncothecaceae), *Pancheria* (Cunoniaceae), *Phyllanthus* (Euphorbiaceae), and *Xylosma* (Flacourtiaceae) (Jaffré, 1979, Reeves, 2003). The most striking of the island’s hyperaccumulators is *Seberita acuminata* (Sapotaceae), a small tree with latex containing over 20% Ni by dry weight (Jaffré *et al.*, 1976). While there have been far fewer hyperaccumulators discovered from the Asian region (Proctor, 2003), there are several that are worthy of mention particularly because these species, or closely-related taxa, are also found in Sri Lanka. For example, Indonesia has yielded Ni hyperaccumulators from the genera *Myristica* (Myristicaceae), *Planchonella* (Sapotaceae), and *Trichospermum* (Tiliaceae). *Rinorea* (Violaceae) is another Asian genus, with two species that hyperaccumulate Ni (Brooks and Wither, 1977a, b; Wither and Brooks, 1977). One of these species, *R. benghalensis*, is known to hyperaccumulate Ni, with up to 1% Ni by dry weight extracted from a herbarium specimen collected from Sri Lanka (Wither and Brooks, 1977). Rajakaruna and Baker (2004) suggest that this species may have been collected from the serpentinized areas of Katupotha in the south-central part of island. The same species was recently collected from Sabah (Jopony and Tongkul, 1999) and confirmed to be a Ni hyperaccumulator. Research conducted on the floras of Sabah and the Philippines (Proctor *et al.*, 1989; Baker *et al.*, 1992) has yielded Ni hyperaccumulators from other genera such as *Brackenridgea* (Ochnaceae), *Dichapetalum*

(Dichapetalaceae), *Walsura* (Meliaceae), as well as *Phyllanthus* (Euphorbiaceae) and *Shorea* (Dipterocarpaceae) widely represented in the Sri Lankan flora. Recent work by Rajakaruna and Bohm (2002) has shown high levels of whole-plant metal accumulation in several Sri Lankan taxa. These include *Crotalaria biflora* (Fabaceae), *Evolvulus alsinoides* (Convolvulaceae), *Hybanthus enneaspermus* (Violaceae) for Ni and *Clerodendrum infortunatum* (Verbenaceae), *Croton bonplandianus* (Euphorbiaceae), *Geniosporum tenuiflorum* (Lamiaceae), *Tephrosia villosa* (Fabaceae), and *Waltheria indica* (Sterculiaceae) for Cu. Copper hyperaccumulation is a rare phenomenon and the taxa shown to accumulate Cu should be carefully examined to further confirm these preliminary findings. This is even more important given Cu is a common contaminant of many metal-enriched habitats and the plants discovered are fast-growing woody plants with large biomass, making them even more suited for phytoremediation. More recently, a thorough examination of plants collected from the Ni- and Cr-enriched serpentine outcrop of Ussangoda (Iqbal *et al.*, 2006), where high Ni-containing taxa were previously discovered (Rajakaruna and Bohm, 2002), has shown Ni hyperaccumulation in *Cassia kleinii* (Fabaceae), a woody shrub well-suited for remediation purposes. Studies by Jayasekera and Rossbach (1994, 1996) also suggest that there may be several taxa in Sri Lanka with the capacity to accumulate high concentrations of heavy metals.

The biological mechanisms for metal hyperaccumulation are varied. A prerequisite for hyperaccumulation, however, is the ability to efficiently tolerate high concentrations of metal within plant tissues. Metal hyperaccumulating species surpass or exceed this prerequisite by having the ability to solubilize metals from the soil, efficiently take up metal using specific ion transporter proteins, and detoxify specific metal effects on cellular processes by chelation and compartmentation, thereby translocating metal even to sensitive regions of the plant, such as leaves, where many important metabolic processes occur (Salt and Krämer, 2000; Reeves and Baker, 2000). The ecological roles of metal hyperaccumulation are still unclear although several studies point to a defense role, *i.e.*, metal-accumulating plants are better able to defend against fungal and insect attacks (Boyd and Martens, 1998). The anti-herbivore and anti-pathogen hypothesis for metal hyperaccumulation has been pursed in some detail over the last few years (Boyd, 2004). What is most interesting about metal hyperaccumulation is that the traits conferring this unusual behavior appear to evolve rapidly in some species within certain families (Bradshaw *et al*., 1990). Given the strong selection pressures imposed by habitats containing metal-enriched soils, it is hardly a surprise to see this level of rapid accommodation in species with the genetic variation required to first colonize contaminated sites. The process of evolution of taxa (*i.e.*, speciation) under extreme geologic conditions, including on soils contaminated due to anthropogenic activities, has been recently reviewed (Rajakaruna and Whitton, 2004).

Physiological, biochemical, and molecular approaches are continually being applied to identify the underlying mechanisms of metal tolerance and hyperaccumulation (Pollard *et al.*, 2002; Lasat, 2002). The drive to find genes underlying these unique biological properties is partly fueled by interest in using transgenic plants in phytoremediation (Cherian and Oliveira, 2005; Pilon-Smits, 2005). Interestingly, as transgenics are being tested in the field and the associated risks assessed, their use appears to be more accepted and less regulated than has been the case for transgenic crops (Pilon-Smits and Pilon, 2002). Research on hyperaccumulator species from the Brassicaceae, including *Allysum bertolonii*, *A. murale*, *Arabidopsis halleri*, *Thlaspi caerulescens* (Chaney *et al*., 2005), and the multi-element accumulator *Brassica juncea*, has greatly benefited from the availability of functional genomics tools already utilized in *Arabidopsis thaliana* research (Cobbett, 2003). Thus, species of Brassicaceae have become model organisms for the molecular genetic analysis of metal hyperaccumulation and tolerance (Palmer *et al*., 2001; Assunção *et al.*, 2003; Peer *et al.*, 2003) and will continue to enrich the field of phytoremediation. Current research suggests that many hyperaccumulators are metal-specific, although several species are able to hyperaccumulate multiple elements (Pollard *et al.*, 2002; Cobbett, 2003). While species able to accumulate one metal are useful for cleaning up areas contaminated with a target metal, those species able to accumulate multiple elements are better suited to deal with most contaminated sites, as contamination is often due to multiple elements and mixtures of organic and inorganic substances (Ensley, 2000).

Despite the discovery of many potential hyperaccumulators, we still do not have adequate information on the distribution of these species in tropical regions or their metal-accumulating behaviors for them to be properly utilized in mineral exploration, phytoremediation, or phytomining (Reeves, 2003; Boominathan *et al.*, 2004). As discussed earlier, success of phytoremediation also relies on the use of species that rapidly produce biomass while also accumulating high concentrations in above-ground tissues. Unfortunately, many of the tropical hyperaccumulators so far discovered are unlikely to be utilized widely for metal clean-up due to their slow growth and herbaceous habit. Attention should be paid to larger woody taxa such as those belonging to genera *Ariadne* (Rubiaceae), *Berkheya* (Asteraceae), *Buxus* (Buxaceae), *Euphorbia* (Euphorbiaceae), *Phyllanthus* (Euphorbiaceae), *Psychotria* (Rubiaceae), and *Rinorea* (Violaceae) (Robinson *et al.*, 1997a; Reeves, 2003; Boominathan *et al*., 2004). These Ni-accumulating woody taxa have the potential for rehabilitating and remediating tropical soils, assuming the soils are contaminated with those metals commonly found in serpentine habitats. The prospective Cu hyperaccumulators recently discovered (Rajakaruna and Bohm, 2002), all with a woody growth habit, could also be employed to remediate Cu-enriched sites in Sri Lanka. Recent studies in Andhra Pradesh, India on *Cassia auriculata* (Fabaceae), *Dodonaea viscose* (Sapindaceae), and *Jatropha curcas* (Euphorbiaceae) suggest the utility of these regionally common species for the reclamation of soils contaminated with a variety of major and trace elements (Nagaraju and Karimulla, 2002).

Leafy vegetables, as well as ornamentals used in the South Asian region, have also shown the capacity to accumulate metals (Bañuelos and Meek, 1989). For example, *Alternanthera philoxeroides*, *A. sessilis*, and *Amaranthus spinosus*, all from Amaranthaceae, growing on metal-enriched sewage sludge in greater Hyderabad City, India, demonstrated the capacity to bioaccumulate Cd, Zn, Fe, and Pb. While extreme caution should be exercised in the use of these plants for human consumption, it is possible that they may be useful in restoring wastelands contaminated with these widespread metals (Prasad and Freitas, 2003). *Amaranthus* species have also been shown to concentrate radionuclides, especially ^{137}Cs, in the shoot (Lasat *et al*., 1998; Dushenkov 2003). Another familiar crop shown to accumulate high concentrations of radionuclides, especially U, is the common sunflower, *Helianthus annuus* (Asteraceae) (Dushenkov *et al*., 1997). This species can also be utilized for the removal of Cd, Cr, and Ni (Chen and Cutright, 2001). *Canna x generalis* (Cannaceae), *Nerium oleander* (Apocynaceae), and *Pelargonium* sp. (Geraniaceae) are some common ornamentals with the capacity to accumulate appreciable quantities of Pb, Cd, and Ni (Saxena *et al*., 1999; Prasad and Freitas, 2003). The finding of high metal concentrations in edible species and in common cultivars suggests that attention should also be paid to potential health risks associated with metal transfer facilitated by such species.

THE NEED FOR FURTHER EXPLORATION AND CONSERVATION

Many hyperaccumulator species or locally-adapted, metal-tolerant populations of these species are rare, often restricted to one or few sites, making the broad collection and utilization of these species difficult. The Ni hyperaccumulator, *Alyssum pinifolium* (Brassicaceae), for example, has been collected only a few times in the last two centuries; this appears to be the case for several hyperaccumulating taxa of this genus (Reeves, 1992). Another Ni hyperaccumulator, *Bornmuellera baldaccii* subsp. *markgrafii* (Brassicaceae) is now represented by a single herbarium specimen (Reeves *et al.*, 1983). It would be a shame if the elusive Sri Lankan Ni hyperaccumulator, *Rinorea benghalensis* (Violaceae) is also limited to a herbarium specimen (Brooks and Withers, 1977a, b). Several Cuban hyperaccumulators are only known from one or two localities or herbarium specimens, including *Phyllanthus grisebachianus* (Euphorbiaceae) now presumed extinct (Reeves and Baker, 2000). While their narrow, highly localized, distributions make these species difficult to find, rarity also raises conservation and management issues that need to be immediately addressed if the species are to be used for phytoremediation purposes. However, it is not only rare taxa that should receive our attention; even widespread, broadly metal-tolerant species should be carefully examined via laboratory screening, as they could harbor locally-adapted, metal-hyperaccumulating populations (Macnair,

2002) in danger of being extirpated. Thus, for conservation of metal tolerance and accumulating traits to be most effective, it is vital that even isolated populations among widespread species be preserved. The knowledge of metal accumulating behavior among tropical taxa is far from adequate to effectively conserve this biological resource of great potential utility. Vigorous research efforts are now needed, especially in under-explored regions such as Sri Lanka, India, and the South Asian region, to enhance our understanding of the extent of metal hyperaccumulation in the local floras. Tracts of land overlying unusual geologies, such as: 1) areas overlying the Cu-Fe mineralization belt along the tectonic boundary between the Vijayan and Highland Series, 2) serpentine outcrops, 3) limestone and other alkaline soils, 4) wastelands resulting from landfills and mining activities, and 5) even areas in the North and East of Sri Lanka contaminated due to over two decades of military activities should be explored carefully as potential sites for harboring this much-valued genetic wealth.

THE TECHNOLOGY OF PHYTOREMEDIATION

Phytoremediation consists of four primary plant-based technologies: 1) rhizofiltration, involving the use of mainly aquatic plants such as *Azolla* spp., *Elodea* spp., *Eichhornia crassipes*, *Lemna* spp., *Myriophyllum* spp., *Typha* spp., and *Vallisneria* spp. to absorb metals and other pollutants from aquatic environments (Zhu *et al*., 1999a; Mahujchariyawong and Ikeda, 2001; Vajpayee *et al*., 2001; Kumar and Chandra, 2004; Liao and Chang, 2004); 2) phytostabilization, involving the use of plants to stabilize and reclaim contaminated terrestrial sites (Berti and Cunningham, 2000); 3) phytovolatilization, involving the use of plants to take-up toxic elements and then convert and release less toxic forms into the atmosphere (Pilon-Smits *et al*., 1999; Lin. *et al*., 2000; Meagher *et al*., 2000; Rugh, 2004); and finally, 4) phytoextraction, the use of plants to absorb metal and other pollutants from soil (Salt *et al.*, 1995; Blaylock and Huang, 2000; Chandra Sekhar *et al*., 2005).

Phytoextraction, perhaps the most widely-utilized method, is most feasible if the plant employed can translocate the metal into its shoots so that the metal can be harvested via phytomining techniques. All phytoremediation techniques are best applied to areas that show low to moderate levels of contamination (Glass, 2000). Further, the depth of soil to be cleaned is determined by the extent of a species' root growth. For many of the currently tested, naturally available hyperaccumulators, this depth is minimal, from a few cm to several m (Schnoor *et al.*, 1995). It is advisable to use native species that grow locally on or near the site; such species are competitive under the local conditions and pose a lesser threat of becoming invasive. Thus, these green technologies should be viewed as long-term, environmentally-friendly methods where best results are obtained only when local species are used, over many seasons, in moderately-contaminated sites, to reduce the metal concentrations to levels that are acceptable for regulatory purposes and for growth of "normal" species. The considerably lower cost and greater social acceptability, however, still make this technology superior to current conventional methods (Wolfe and Bjornstad, 2002). Detailed studies on propagation, planting densities, growth rates, optimum soil amendments, watering regimes, control of insects and disease, and harvesting methods are currently being carried out (Chaney *et al.*, 2000; Angle *et al.*, 2001) to determine the efficiency of metal removal for temperate hyperaccumulator species such as *Alyssum bertolonii* (Brassicaceae) and *Streptanthus polygaloides* (Brassicaceae) (Robinson *et al*., 1997b; Nicks and Chambers, 1998; Brooks *et al*., 2001) and should provide insightful guidelines for any study seeking to use tropical accumulators for soil remediation or phytomining (Reeves, 2003).

One of the most promising and well-tested candidates for phytoextraction is a plant familiar to many Sri Lankans, the Indian Mustard, *Brassica juncea* (Brassicaceae). This yellow-flowered, weedy mustard is cultivated or naturalized in many parts of the world, extending from India through western Egypt and Central Asia to Europe (Prasad and Freitas, 2003). The species is capable of producing 18 tons of biomass per hectare per crop and is able to simultaneously accumulate and translocate high levels of Cu, Cr, Cd, Ni, Pb, and Zn to the fast-growing shoot system (Kumar *et al*., 1995; Blaylock *et al*., 1997; Begonia *et al*., 1998, Zhu *et al*., 1999b). Several studies have examined the nature of metal accumulation (Qadir *et al*., 2004), including the uptake of radionuclides such as U and ^{137}Cs, in this wonder-crop (Huang *et al*., 1998; Lasat *et al*., 1998; Dushenkov, 2003) while others have successfully used the species in field remediation trials even in the United States (Tucker and Shaw, 2000). The USDA-ARS Plant Introduction Station at Iowa State University, Ames, Iowa (http://www.ars.usda.gov/Main/) harbors a worldwide collection of *B. juncea* accessions with demonstrated capacity to accumulate metal and other pollutants. Seeds are distributed for public and private research purposes at no cost.

LIMITATIONS AND CONCERNS OF PHYTOREMEDIATION

Baker and Whiting (2002) suggest that while the discovery of metal hyperaccumulators has clearly gained momentum by the "hype" of exploiting these species in phytoremediation-phytomining operations, caution must also be exercised in the application of this knowledge. First, there appears to be a widening gap between the science and application (Ernst, 2005); there are surprisingly few applications of metal-accumulating plants for remediation or metal-recovery purposes (Chaney *et al*., 2000; Li *et al*., 2003). Second, considerable expectations are now placed on genetic modification to generate model plants for commercial metal-extraction (Raskin, 1996; Rugh, 2004). Although modification can produce idealized hyperaccumulators, increasing public concern over the utilization of genetically-modified organisms could force governments to prohibit their use. While it is sensible to explore genetic modification for metal clean-up in the case of Hg (Pilon-Smits and Pilon, 2000) and other elements plants are unable to naturally hyperaccumulate (Moffat, 1999), every effort should be made to search for "natural" hyperaccumulators, especially from under-explored metal-enriched sites. Encouraging rigorous botanical explorations worldwide will clearly contribute to cataloging and conserving the unique plant diversity found in environments that are metal-enriched due to natural or anthropogenic causes (Whiting *et al.*, 2002; Whiting *et al.*, 2004) leading to a larger knowledge base and germ bank of tropical hyperaccumulators that can be utilized effectively in tropical regions where phytoremediation technologies are most-needed (Prasad and Freitas, 2003). Further, conventional plant breeding practices should always be explored to create the idealized hyperaccumulator prior to utilizing invasive genetic manipulations (Baker and Whiting, 2002).

Ernst (2000; 2005) also warns that phyto-remedation is only a "hype" and up to now phytoextraction of heavy metals has been nothing more than transporting the harvest of metal-loaded plants from contaminated to clean sites (note, however, that landfills that harbor waste generated from technological means are generally previously uncontaminated sites now containing pollutants). Hence, until phytoremediation efforts are closely-linked to phytomining operations, the extracted metal has to be released back into the environment, although the area of contamination can be significantly reduced if the metal-enriched material is ashed and properly discarded. For example, biomass that contains extracted Se, an essential nutrient, can be transported to areas deficient in this element and used for animal feed (Bañuelos and Meek, 1989). Highly productive and almost cosmopolitan crops like corn (*Zea mays*), in combination with metal tolerant arbuscular mycorrhizal fungi, are also able to remediate soils slightly contaminated with heavy metals (Florijn and Van Beusichem, 1993; Hildebrandt *et al*., 1999). In cases where local hyperaccumulators are unavailable, species such as corn may be a preferred choice over transgenic plants (Eapen and D'Souza, 2005) to clean up low levels of metal contamination (Ernst, 2000).

Phytoremediation has several limitations that should also be considered prior to employing plants to remediate a site (Chaudhry *et al*., 1998; Angle and Linacre, 2005). Soil properties, contaminant toxicity level, and climate must allow the selected species to grow. This is especially important when local accumulator species are lacking and one is forced to bring in "alien" hyperaccumulators. Phytoremediation is also often slower in achieving the anticipated result than traditional remediation methods such as excavation, incineration, and pump-and-treat systems (Glass, 1999). The time factor is clearly the biggest weakness of phytoremediation. Phytoremediation is also limited by the bioavailability of the pollutant. If only a fraction of the pollutant is bioavailable, but the regulatory clean up standards require that all of the pollutant be removed, then "green clean" is not sufficient. In such cases, bioavailability may be enhanced via soil amendments (Salt *et al*., 1998) or engineering-based technologies to enhance the efforts of the biological method. Such an integrated remediation effort requires a multidisciplinary team of scientists; a set of skills and expertise that may not always be locally available in some developing countries.

Although it is tempting to incorporate low impact, solar-driven phytoremediation technologies to resolve environmental concerns, it is critical that we appreciate that the underlying biological mechanisms of hyperaccumulators and their interactions with associated biota remain largely unknown. Thus, to enhance the efficiency of phytoremediation it is critical that we examine in greater detail the biological processes and ecological consequences of employing plants to clean-up the environment. In addition to the immediate need to better understand species-specific physiological processes such as ion uptake, translocation, tolerance, and hyperaccumulation, close attention should also be given to plant-microbe interactions and other rhizospheric processes that contribute to efficient uptake. Further, the movement of pollutants via plants to higher trophic levels should be carefully studied since phytoremediation clearly has the potential to enhance metal transfer through the food chain (Wall and Boyd, 2002; Peterson *et al.*, 2003) causing a significant health concern in areas where this technology is employed.

CONCLUSIONS

In the past decade phytoremediation has gained wide acceptance as an effective green technology as well as a rigorous field of research. Industrial and academic researchers have come together to explore the multi-faceted nature of the fascinating biological phenomenon of metal hyperaccumulation, making the field of phytoremediation truly interdisciplinary and collaborative. The field continues to benefit from this approach, involving research teams at all organization levels studying the remediation of pollutants from the molecule to the ecosystem. Several journals are devoted to publishing research from this highly interdisciplinary field. Among them are the *Journal of Environmental Quality*, *International Journal of Phytoremediation*, *Journal of Geochemical Exploration*, and *Mining Environmental Management*. Currently, there are online databases such as PHYTOPET and PHYTOREM listing information on plant species useful for remediating sites with a range of pollutants (McIntyre, 2003). The US Environmental Protection Agency (EPA) also maintains a website for researchers and the general public on the growing field of phytoremediation (http://www.clu-in.org).

If phytoremediation is to succeed in all parts of the world, areas rich in heavy metals and other pollutants have to be explored in greater detail in order to discover the “raw” material required for phytoremediation. In Sri Lanka and other regions of South Asia there are mine and smelter wastes enriched in Pb, Zn, Cd, Cu, Fe, Co, and As. In many such sites detailed biogeochemical work has not yet been done. Similarly, areas overlying outcroppings of unusual geologies must be subjected to detailed botanical collections and plant-soil analyses. In many instances where such efforts have been undertaken, novel biological entities or unusual biological phenomena have been discovered (Reeves, 2003). Thus, field exploration of unusual geologies and contaminated sites in Sri Lanka and the adjacent region should become a priority (Jayasekera and Rossbach, 1993). Funding should also be made available for research and the training of personnel in the many areas surrounding phytoremediation. In 1994 Sri Lanka ratified the Convention on Biological Diversity, committing itself via this international treaty to preserving global and national biodiversity, especially rare and endemic species, for the benefit of mankind. Metal hyperaccumulating species should be considered as biodiversity assets covered by this treaty. Such approaches will help achieve the maximum potential in the collection, use, and conservation of hyperaccumulating species for phytoremediation-phytomining operations. This is especially important since it is clear that the greatest application of phytoremediation will be in developing countries such as Sri Lanka, where this technology can provide a low-cost means of controlling widespread environmental contamination.

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REFERENCES

Adriano, D. C. (2001). *Trace Elements in the Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals*. Springer, New York, USA. 866 p.

Allinson, G., Nishikawa, M., De Silva, D. D., Laurenson, L. J. B. and De Silva, K. (2002). Observations on metal concentration in Tilapia (*Oreochromis mossambicus*) in reservoirs of South Sri Lanka. *Ecotoxocology and Environmental Safety* **51**: 197-202.

Anderson, C. W. N., Brooks, R. R., Stewart, R. B. and Simcock, R. (1998). Induced hyperaccumulation of gold in plants. *Nature* **395**: 553-554.

Anderson, C. W. N., Brooks, R. R., Chiarucci, A., LaCoste, C. J., Leblanc, M., Robinson, B. H., Simcock, R. and Stewart, R. B. (1999). Phytomining for nickel, thallium, and gold. *Journal of Geochemical Exploration* **67**: 407-415.

Anderson, C. W. N., Moreno, F. and Meech, J. (2005). A field demonstration of gold phytoextraction technology. *Minerals Engineering* **18**: 385-592.

Angle, J. S., Chaney, R. L., Baker, A. J. M, Li, Y., Reeves, R., Volk, V., Roseberg, R., Brewer E., Burke S. and Nelkin J. (2001). Developing commercial phytoextraction technologies: practical considerations. *South African Journal of Science* **97**: 619-623.

Angle J. S. and Linacre, N. A. (2005). Metal phytoextraction—A survey of potential risks. *International Journal of Phytoremediation* **7**: 241-254.

Aprill, W. and Sims, R. C. (1990). Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere* **20**: 253-265.

Assunção, A. G. L., Schat, H. and Aarts, M. G. M. (2003). *Thlaspi caerulescens*, the ideal model species to study heavy metal hyperaccumulation in plants. *New Phytologist* **159**: 351-360.

Baker, A. J. M., Proctor, J. and Reeves, R. D. (1992). *The Vegetation of Ultramafic (Serpentine) Soils.* Proceedings of the First International Conference on Serpentine Ecology, University of California, Davis, 19-22 June 1991. Intercept, Hampshire, UK. 509 p.

Baker, A. J. M., McGrath, S.P., Reeves, R.D. and Smith, J.A.C. (2000). Metal hyperaccumlator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal polluted soils. In: N. Terry and G. Bañuelos (Eds.), *Phytoremediation of Contaminated Soil and Water,* Lewis, Boca Raton, FL, USA: 85-108.

Baker, A. J. M and Whiting, S. M. (2002). In Search for the Holy Grail—another step in understanding metal hyperaccumulation? *New Phytologist* **155**: 1-7.

Bandara, N. I. G. J. (2003). Water and wastewater issues in Sri Lanka. *Water Science and Technology* **47**: 305-312.

Banks, D., Younger, P. L., Arnesen, R. T., Iversen, E. R. and Banks, S. (1997). Mine-water chemistry: the good, the bad, and the ugly. *Environmental Geology* **32**: 157-174.

Bañuelos, G. S. and Meek D. W. (1989). Selenium accumulation in selected vegetables. *Journal of Plant Nutrition* **12**: 1255-1272.

Begonia, G. B., Davis, C. D., Begonia, M. E. T. and Gray C. N. (1998). Growth responses of Indian Mustard (*Brassica juncea* (L.) Czern.) and its phytoextraction of lead from a contaminated soil. *Bulletin of Environmental Contamination and Toxicology* **61**: 38-43.

Berti, W. R. and Cunningham, S. D. 2000. Phytostabilization of metals. In: *Phytoremediation of toxic metals. Using plants to clean up the environment,* I. Raskin and B. D. Ensley (Eds.), J. Wiley & Sons, New York, USA: 71-88.

Blaylock, M. J., Salt, D. E., Dushenkov, S. Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D. and Raskin I. (1997). Enhanced accumulation of Pb in Indian Mustard by soil-applied chelating agents. *Environmental Science and Technology* **31**: 860-856.

Blaylock, M. J. and Huang, J. W. (2000). Phytoextraction of metals. In: I. Raskin and B. D. Ensley (Eds.), *Phytoremediation of toxic metals. Using plants to clean up the environment,* J. Wiley & Sons, New York, USA: 53-70.

Boominathan, R., Saha-Chaudhury, N. M., Sahajwalla, V. and Doran, P.M. (2004). Production of nickel bio-ore from hyperaccumlator plant biomass: Applications in phytomining. *Biotechnology and Bioengineering* **86**: 243-250.

Boyd, R. S. and Martens, S. N. (1998). The significance of metal hyperaccumulation for biotic interactions. *Chemoecology* **8:** 1-7.

Boyd, R. S. (2004). Ecology of metal hyperaccumulation. *New Phytologist* **162**: 563-567.

Bradshaw, A. D., McNeilly, T. and Putwain, P. D. (1990). The essential qualities. In: *Heavy Metal Tolerance in Plants: Evolutionary Aspects,* A. J. Shaw (Ed.), CRC Press, Boca Raton, FL, USA. Pp 323-334.

Bridge, G. (2004). Contested terrain: mining and the environment. *Annual Review of Environment and Resources* **29**: 205-259.

Brooks, R. R. and Wither, E. D. (1977a). Nickel hyperaccumulation by *Rinorea bengalenis* (Wall.) O.K. *Journal of Geochemical Exploration* **7**: 295-300.

Brooks R. R. and Wither E. D. (1977b). Cobalt and nickel in *Rinorea* species. *Plant and Soil* **47**: 707-712

Brooks, R. R., Chambers, M. F., Nicks, L. J. and Robinson B.H. (1998). Phytomining. *Trends in Plant Science* **3**: 359-362.

Brooks, R.R., Robinson, B.H., Howes, A.W. and Chiarucci, A. (2001). An evaluation of *Berkheya coddii* Roessler and *Alyssum bertolonii* Desv. for phytoremediation and phytomining of nickel. *South African Journal of Science* **97**: 558-560.

Burken, J. G. and Schnoor, J. L. (1997). Uptake and metabolism of atrazine by poplar trees. *Environmental Science and Technology* **31**: 1399-1406.

Chandra Sekhar K., Kamala, C. T., Chary, N. S. Balaram, V. and Garcia, G. (2005). Potential of *Hemidesmus indicus* for phytoextraction of lead from industrially contaminated soils. *Chemosphere* **58**: 507-514

Chaney, R. L., Malik, M., Li, Y. M., Brown, S. L., Brewer, E. P., Angle, J. S. and Baker, A. J. M. (1997). Phytoremediation of soil metals. *Current Opinion in Biotechnology* **8**: 279-284.

Chaney, R. L., Li, Y. M., Brown, S. L., Homer, F. A. and Malik, M. (2000). Improving metal hyperaccumulator wild plants to commercial phytoextraction systems: approaches and progress. In: N. Terry and G. Bañuelos (Eds.), *Phytoremediation of Contaminated Soil and Water,* Lewis, Boca Raton, FL, USA: 129-158.

Chaney, R. L., Angle, J. S., McIntosh, M. S., Reeves, R. D., Li, Y. M., Brewer, E. P., Chen, K. Y., Roseberg, R. J., Perner, H., Synkowski, E. C., Broadhurst, C. L., Wang, S. and Baker A. J. M. (2005). Using hyperaccumulator plants to phytoextract soil Ni and Cd. *Zeitschrift fuer Naturforschung Section C Journal of Biosciences* **60**: 190-198.

Chaudhry, T. M., Hayes, W. J., Khan, A.G. and Khoo C.S. (1998). Phytoremediation—focusing on accumulator plants that remediate metal-contaminated soils. *Australian Journal of Ecotoxicology* **4**: 27-51.

Chen, H. and Cutright, T. (2001). EDTA and HEDTA effects on Cd, Cr, and Ni uptake by *Helianthus annuus. Chemosphere* **45**: 21-28.

Cherian, S. and Oliveira, M. M. (2005). Transgenic plants in phytoremediation: Recent advances and new possibilities. *Environmental Science and Technology* **39**: 9377-9390.

Cobbett, C. (2003). Heavy metals and plants—model systems and hyperaccumulators. *New Phytologist* **159**: 289-293.

Cunningham, S. D. and Ow, D. W. (1996). Promises and prospects of phytoremediation. *Plant Physiology* **110**: 715-719.

Dissanayake, U. S., Tennakoon, K. U. and Priyantha, N. (2002). Potential of two invasive plant species, *Lantana camara* L. and *Wedelia trilobata* L., for selective heavy metal uptake. *Ceylon Journal of Science (Bio. Sci.)* **29**: 1-11.

Dushenkov, S., Vasudev, D., Kapulnik, Y., Gleba, D., Fleisher, D., Ting, K. C. and Ensley, B. D. (1997). Removal of uranium from water using terrestrial plants. *Environmental Science and Technology* **31**: 3468-3474.

Dushenkov, S. (2003). Trends in phytoremediation of radionuclides. *Plant and Soil* **249**: 167-175.

Eapen, S. and D'Souza, S. F. (2005). Prospects of genetic engineering of plants for phytoremediation of toxic metals. *Biotechnology Advances* **23**: 97-114.

Ensley, B. D. (2000). Rationale for use of phytoremediation. In: I. Raskin and B. D. Ensley (Eds.), *Phytoremediation of Toxic Metals. Using Plants to Clean up the Environment,* J. Wiley & Sons, New York, USA: 3-11.

Ernst, W. H. O. (2000). Evolution of metal hyperaccumulation and the phytoremediation hype. *New Phytologist* **146**: 357-58.

Ernst, W. H. O. (2005). Phytoextraction of mine wastes—Options and impossibilities. *Chemie der Erde Geochemistry* **65**: 29-42.

Florijn, P. J. and Van Beusichem, M. L. (1993). Uptake and distribution of cadmium in maize inbred lines. *Plant and Soil* **150**: 25-32.

Ghosh, M. and Singh, S. P. (2005). A review of phytoremediation of heavy metals and utilization of its byproducts. *Applied Ecology and Environmental Research* **3**: 1-18.

Glass, D. J. (1999). *US and International Markets for Phytoremediation, 1999-2000*. D. Glass Associates, Inc, Needham, MA, USA. 270 p.

Glass, D. J. (2000). Economic potential of phytoremediation. In: I. Raskin and B. D. Ensley, (Eds.), *Phytoremediation of Toxic Metals. Using Plants to Clean up the Environment,* J. Wiley & Sons, New York, USA: 15-31.

Grêman, H., Vodnik, D., Velikonja-Bolta, Š., and Leštan, D. (2003). Heavy metals in the environment. *Journal of Environmental Quality* **32**: 500-506.

Harms, H., Bokern, M., Kolb, M. and Bock, C. (2003). Transformation of organic contaminants by different plant systems. In: S. C. McCutcheon and J. L. Schnoor (Eds.), *Phytoremediation: Transformation and Control of Contaminants,* Wiley and Sons, New York, USA: 285-316.

Hildebrandt, U., Kaldorf, M. and Bothe, H. (1999). The zinc violet and its colonization by arbuscular mycorrhizal fungi. *Journal of Plant Physiology* **154**: 709-717.

Hong, M. S., Farmayan, W. F., Dortch, I. J., Chiang, C. Y., McMillan, S. K. and Schnoor, J. L. (2001). Phytoremediation of MTBE from groundwater plume. *Environmental Science and Technology* **35**: 1231-1239.

Huang, J. W., Blaylock, M. J., Kapulnik, Y. and Ensley, B. D. (1998). Phytoremediation of uranium-contaminated soils: role of organic acids in triggering uranium hyperaccumulation in plants. *Environmental Science and Technology* **32**: 2004-2008.

Hughes, J. B., Shanks J., Vanderford M., Lauritzen J. and Bhadra, R. (1997). Transformation of TNT by aquatic plants and plant tissue cultures. *Envrionmental Science and Technology* **31**: 266-71.

Iqbal, M. C. M., Kulasekara, L., Rajakaruna, N. and Iqbal, S. S. (2006). Plant-soil relations of a serpentine site in the southern coast of Sri Lanka. *Fifth International Conference on Serpentine Ecology*. Siena, Italy, 09-13 May. (Abstract).

Jaffré, T., Brooks R.R., Lee J. and Reeves R.D. (1976). *Seberita acuminata*: a hyperaccumlator of nickel from New Caledonia. *Science* **193**: 579-580.

Jaffré, T., Kersten, W., Brooks, R. R. and Reeves, R. D. (1979). Nickel uptake by Flacourtiaceae of New Caledonia. *Proceedings of the Royal Society of London* **B205**: 385-394.

Jopony, M. and Tongkul F. (1999). Hyperaccumulation of nickel by *Rinorea benghalensis* in ultrabasic soils of Sabah, Malaysia, *Third International Conference on Environmental Chemistry and Geochemistry in the Tropics*. Hong Kong, 24-26 November. Abstract.

Jayasekera, R. and Rossbach, M. (1993). Implementation of specimen banking concepts in developing countries: first trial – Sri Lanka. *The Science of the Total Environment* **139/140**: 139-142.

Jayasekera, R. and Rossbach, M. (1994). Trace elements in plants growing in contrasting ecosystems in Sri Lanka: analytical aspects. *Environmental Geochemistry and Health* **16**: 171-179.

Jayasekera, R. and Rossbach, M. (1996). Background levels of heavy metals in plants of different taxonomic groups from a montane rain forest in Sri Lanka. *Environmental Geochemistry and Health* **18**: 55-62.

Krämer, U. (2003). Phytoremediation to phytochelatin—plant trace metal homeostasis. *New Phytologist* **158**: 4-6.

Kumar, P. B. A. N., Dushenkov, V., Motto, H. and Raskin, I. (1995). 1. Phytoextraction: the use of plants to remove heavy metals form soils. *Environmental Science and Technology* **29**: 1232-1238.

Kumar, P. and Chandra, R. (2004). Detoxification of distillery effluent through *Bacillus thuringiensis* (MTCC 4714) enhanced phytoremediation potential of *Spriodela polyrrhiza* (L.) Schliden. *Bulletin of Environmental Contamination and Toxicology* **73**:903-910.

Lasat, M. M., Fuhrmann, M., Ebbs, S. D., Cornish, J. E. and Kochian, L. V. (1998). Phytoremediation of a radiocesium-contaminated soil: evaluation of cesium-137 bioaccumulation in the shoots of three plant species. *Journal of Environmental Quality* **27**: 165-169.

Lasat, M. M. (2002). Phytoextraction of toxic metals: A review of biological mechanisims. *Journal of Environmental Quality* **31**: 109-120.

Li, Y. M., Chaney, R., Brewer, E., Rosenberg, R., Angle, S.J., Baker, A. J. M., Reeves, R. D. and Nelkin, J. (2003). Development of technology for commercial phytoextraction of nickel: economic and technical considerations. *Plant and Soil* **249**: 107-115.

Liao, S.W. and Chang, W.L. (2004). Heavy metal phytoremediation by Water Hyacinth at constructed wetlands in Taiwan. *Journal of Aquatic Plant Management* **42**: 60-68.

Lin Z. Q., Schemenauer R. S., Cervinka, V., Zayed, A., Lee, A. and Terry, N. (2000). Selenium volatilization from a soil-plant system for the remediation of contaminated water and soil in the San Joaquin Valley. *Journal of Environmental Quality* **29:** 1048-1056.

Macnair, M. R. (2002). Within-and-between-population genetic variation for zinc accumulation in *Arabidopsis halleri*. *New Phytologist* **155**: 59-66.

Mahujchariyawong, J. and Ikeda S. (2001). Modelling of environmental phytoremediation in the eurtrophic river—the case of water hyacinth harvest in Tha-chin River, Thailand. *Ecological Modelling* **142**: 121-134.

McIntyre, T. C. (2003). Databases and protocol for plant and microorganism selection: hydrocarbons and metals. In:, S.C. McCutcheon and J.L. Schnoor (Eds.), *Phytoremediation, Transformation, and Control of Contaminates* Wiley & Sons, New York, NY, USA: 887-904.

Meagher, R. B., Rugh, C. L., Kandasamy ,M. K., Gragson, G. and Wang, N. J. (2000). Engineered phytoremediation of mercury pollution in soil and water using bacterial genes. In: N. Terry and G. Banuelos (Eds.), *Phytoremediation of Contaminated Soil and Water*, Lewis, Boca Raton, FL, USA: 201-221.

Meharg, A. A. (2002). Arsenic and old plants. *New Phytologist* **156**: 1-4.

Moffat, A. S. (1999). Engineering plants to cope with metals. *Science* **285**: 369-370.

Mohan, B. S. (2005). Phytomining of Gold. *Current Science* **88**: 1021-1022.

Nagaraju, A. and Karimulla S. (2002). Accumulation of elements in plants and soils in and around Nellore mica belt, Andhra Pradesh, India-A biogeochemical study. *Environmental Geology* **41**: 852-860.

Newman, L. A., Strand, S. E., Choe, N., Duffy, J., Ekuan, G. Ruszaj, M., Shurtleff, B. B., Wilmoth, J., Heilman, P. and Gordon, M. P. (1997). Upake and biotransformation of trichloroethylene by hybrid poplars. *Environmental Science and Technology* **31**: 1062-1067.

Nicks, L. J. and Chambers M. F. (1998). A pioneering study of the potential of phytomining for nickel. In: R. R. Brooks (Ed.), *Plants that Hyperaccumulate Heavy Metals,* CAB International, Wallingford, New Zealand: 313-326.

Nriagu, J. O. (1979). Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. *Nature* **279**: 409-411.

Palmer, E. F., Warwick, F. and Keller, W. (2001). Brassicaceae (Cruciferae) family, plant biotechnology and phytoremediation. *International Journal of Phytoremediation* **3**: 245-287.

Peer, W. A., Mamoudian, M., Lahner, B., Reeves, R. D., Murphy, A. S. and Salt, D. E. (2003). Development of a model plant to study the molecular genetics of metal hyperaccumulation. Part I: Germplasm analysis of 20 Brassicaceae accessions from Austria, France, Turkey, and USA. *New Phytologist* **159**: 421-430.

Peterson, L. R., Trivett, V., Baker, A. J. M., Aguiar, C. and Pollard J.A. (2003). Spread of metals through an invertebrate food chain as influenced by a plant that hyperaccumulates nickel. *Chemoecology* **13**: 103-108.

Petrisor, I. G. Dobrota S., Komitsas K., Lazar I., Kuperberg J. M, Serban M. (2004). Artificial Inoculation- Perspectives in Tailings Phytostabilization. *International Journal of Phytoremediation* **6**: 1-15.

Pilon-Smits, E. A. H., de Souza, M. P., Hong G., Amini A., Bravo R. C., Payabyab, S. T. and Terry, N. (1999). Selenium volatilization and accumulation by twenty aquatic plant species. *Journal of Environmental Quality* **28**: 1011-1017.

Pilon-Smits, E. A. H. and Pilon M. (2000). Breeding mercury-breathing plants for environmental cleanup. *Trends in Plant Science* **6**: 235-236.

Pilon-Smits, E. A. H. and Pilon, M. (2002). Phytoremediation of metals using transgenic plants. *Critical Reviews in Plant Sciences* **21**: 439-456.

Pilon-Smits, E. A. H. (2005). Phytoremediation. *Annual Review of Plant Biology* **56**: 15-39

Pollard, A. J., Powell, K. D., Harper, F. A., and Smith, J. A. C. (2002). The genetic basis for metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences* **21**: 539-566.

Prasad, M. N. V. and Freitas, H. M. O. (2003). Metal hyperaccumulation in plants—Biodiversity prospecting for phytoremediation technology. *Electronic Journal of Biotechnology* **6**: 285-321.

Proctor, J., Phillipps, C., Duff, G. K., Heaney, A. and Robertson, F. M. (1989). Ecological studies in Gunung Silam, a small ultrabasic mountain in Sabah, Malaysia. II. Some forest processes. *Journal of Ecology* **77**: 317-331.

Proctor, J. (2003). Vegetation and soil and plant chemistry on ultramafic rocks in the tropical Far East. *Perspectives in Plant Ecology, Evolution, and Systematics* **6**: 105-124.

Qadir, S., Qureshi, M. I., Javed, S. and Abdin, M. Z. (2004). Genotypic variation in phytoremediation potential of *Brassica juncea* cultivars exposed to Cd stress. *Plant Science* **167**: 1171-1181.

Rajakaruna, N. and Bohm, B. A. (2002). Serpentine and its vegetation: a preliminary study from Sri Lanka. *Journal of Applied Botany* **76**: 20-28.

Rajakaruna, N. and Baker, A. J. M. (2004). Serpentine: A model habitat for botanical research in Sri Lanka. *Ceylon Journal of Science (Biological Sciences)* **32**: 1-19.

Rajakaruna, N. and Whitton, J. (2004). Trends in the evolution edaphic species with an example of parallel evolution in the *Lasthenia californica* complex. In: Q.C.B. Cronk, I.E.P. Taylor, R. Ree, and J. Whitton (Eds.), *Plant adaptation: Molecular*

Biology and Ecology, NRC Research Press, Ottawa, Ontario, Canada: 103-110.

Raskin, I. (1996). Plant genetic engineering may help with environmental cleanup. *Proceedings of National Academy of Sciences* **93**: 3164-3166.

Raskin, I. and Ensley, B .D. (2000). *Phytoremediation of toxic metals. Using plants to clean up the environment.* J. Wiley & Sons, New York, USA. 304 p.

Reeves, R. D., Brooks, R. R. and Dudley, T. R. (1983). Uptake of nickel by species of *Alyssum*, *Bornmuellera* and other genera of Old World Tribus Alysseae. *Taxon* **32**: 184-192.

Reeves, R. D. (1992). The hyperaccumulation of nickel by serpentine plants. In: A. J. M. Baker, R. D. Reeves, and J. Proctor (Eds.), *The vegetation of ultramafic (serpentine) soils*,Intercept, Andover Hants, UK: 253-277.

Reeves, R. D. and Baker, A. J. M (2000). Metal-Accumulating Plants. In: I. Raskin and B. D. Ensley (Eds.), *Phytoremediation of toxic metals. Using plants to clean up the environment*, J. Wiley & Sons, New York, USA: 193-229.

Reeves, R. D. (2003). Tropical hyperaccumulators of metals and their potential for phytoextraction. *Plant and Soil* **249**: 57-65.

Robinson, B. H., Brooks, R. R., Howes, A.W., Kirkman, J. H. and Gregg, P. E. H. (1997a). The potential of high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phyto-mining. *Journal of Geochemical Exploration* **60**: 115-126.

Robinson, B. H., Chiarucci, A., Brooks, R. R., Petit, D., Kirkmam, J. H., Gregg, P. E. H. and De Dominicis, V. (1997b). The nickel hyperaccumu-mulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel. *Journal of Geochemical Exploration* **60**: 115-126.

Robinson, B. H., Fernandez, J. E., Madejon, P., Maranon, P., Murillo, J.M., Green, S. and Clothier, B. (2003a). Phytoextraction: an assessment of biogeochemical and economic viability. *Plant and Soil* **249**: 117-125.

Robinson, B. H., Green, S., Mills T., Clothier, B., Velde, M, Laplane, R., Fung, L., Deurer, M., Hurst, S., Thayalakumaran, T. and Dijssel, C. (2003b). Phytoremediation: using plants as biopumps to improve degraded environments. *Australian Journal of Soil Research* **41**: 599-611.

Rugh, C. L. (2004). Genetically engineered phytoremediation: one man's trash is another man's transgene. *Trends in Biotechnology* **22**: 496-468.

Salt D. E., Blaylock M., Kumar, N. P. B. A., Dushenkov, V., Ensley, B. D., Chet, I. and Raskin, I. (1995). Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology* **13**: 468-474.

Salt, D. E., Smith, R. D. and Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 643-668.

Salt, D. and Krämer, U. (2000). Mechanisims of metal hyperaccumulation in plants. In: *I*. Raskin and B. D. Ensley (Eds.), *Phytoremediation of toxic metals. Using plants to clean up the environment*, Wiley & Sons, New York, USA: 231-246.

Saxena, P. K., Krishnara, J., Dan, T., Perras, M. R. and Vettakkoruma-Kankav, N. N. (1999). Phytoremediation of metal contaminated and polluted soils. In: M. N. V. Prasad and J. Hagemeyer (Eds.), *Heavy metal stress in plants - From molecules to ecosystems.* Springer-Verlag, Heidelberg, Germany: 305-329.

Schnoor, J. L., Light, L. A., Mccutcheon, S. C., Wolfe, N. L. and Carreira, L. H. (1995). Phytoremediation of organic and nutrient contaminants. *Environmental Science and Technology* **29**: 318-323.

Tsao, D. T. (2003). Phytoremediation. *Advances in Biochemical Engineering Biotechnology* 78. Springer-Verlag, Berlin, Germany. 206 p.

Tucker, R. K. and Shaw J.A. (2000). Phytoremediation and public acceptance. In: I. Raskin and B. D. Ensley (Eds.), *Phytoremediation of toxic metals. Using plants to clean up the environment*, J. Wiley & Sons, New York, USA: 33-42.

Vajpayee, P., Rai, U. N., Ali, B., Tripathi, R. D., Yadav, V., Sinha, S. and Singh S. N. (2001). Chromium-Induced Physiologic changes in *Vallesneria spiralis* L. and its role in phytoremediation of tannery effluent. *Bulletin of Environmental Contamination and Toxicology* **67**: 246-256.

Visoottiviseth, P., Francesconi, K. and Sridokchan, W. (2002). The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. *Environmental Pollution* **118**: 453-461.

Wall, M. A. and Boyd, R. C. (2002). Nickel accumulation in serpentine Arthropods from the Red Hills, California. *Pan-Pacific Entomologist* **78**: 168-176.

Whiting, S. N., Reeves, R. D. and Baker, A. J. M. (2002). Conserving biodiversity: Mining metallophytes and reclamation. *Mining Environmental Management* **10**: 11-16.

Whiting, S. N., Reeves, R. D., Richards, D., Johnson, M. S., Cooke, J. A., Malaisee, F., Paton, A., Smith, J. A. C., Angle, J. S., Chaney, R. L., Ginoocchio, R., Jaffré, T., Johns, R., McIntyre, T., Purvis, O. W., Salt, D. E., Schat, H., Zhao, F. J. and Baker, A. J. M. (2004). Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. *Restoration Ecology* **12**: 106-116.

Wither E. D. and Brooks R. R. (1977). Hyperacumulation of nickel by some plants of Southeast Asia. *Journal of Geochemical Exploration* **8**: 579-583.

Wolfe, A. K. and Bjornstad, D. J. (2002). Why would anyone object? An exploration of social aspects of phytoremediation acceptability. *Critical Reviews in Plant Science* **21**: 429-438.

World Health Organization (2003). Health as a cross-cutting issue in dialogues on water for food and the environment: Report of an international workshop. http://whqlibdoc.who.int/hq/2004/WHO_SDE_WSH_04.02.pdf.

Zhu, Y. L., Zayed A. M., Quian J. H., DeSouza, M. and Terry N. (1999a). Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. *Journal of Environmental Quality* **28**: 339-344.

Zhu, Y. L., Pilon-Smits, E. A. H., Jouanin, L. and Terry, N. (1999b). Overexpression of gluthathione synthetase in *Brassica juncea* enhances cadmium tolerance and accumulation. *Plant Physiology* **119**: 73-79.