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Scented Geraniums: a Model System for Phytoremediation

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ABSTRACT All living organisms depend on soil and water for their sustained growth and development. In recent years, sustenance of life in these growth matrices has been adversely affected by the cumulative increase in environmental pollutants resulting from increasing population, growing economies and resource-use. This review provides a glimpse into the problem of global environmental pollution, the traditional technologies available for remediation and the scope of emerging 'plant-based remediation' technologies. Phytoremediation, the use of plants to effectively remove or stabilize contaminants from the growth substrate, is a low cost and ecologically friendly alternative to the common 'dig and dump' technologies. The field of phytoremediation has been driven by the intrinsic need for identification of ideal candidate plant species. To date, there are only a very few identified plants which satisfy all of the prerequisites for use in phytoremediation. The review focuses on one such plant species, the common horticultural plant scented geranium (*Pelargonium* sp.), with demonstrated potential to remediate metal / salt contaminated soils / aqueous systems. The characterization of tolerance and metal / salt accumulation potential of *Pelargonium* sp. and its efficacy in remediating complex contaminated sites are described. The unique ability of scented geraniums to tolerate excessive amounts of multi-metals, hydrocarbon and salt mixtures, and at the same time to accumulate significant amounts of metal and salt ions in the biomass, renders this plant species as one of the ideal candidates for remediation.

Key words: Metal accumulation, metal tolerance, *Pelargonium* sp., phytoremediation, soil remediation

Introduction

All living organisms (plants, animals, including humans) depend on soil, water and air for their sustained growth and development. In many instances the sustenance of life in the growth matrix is adversely affected by the presence of deleterious substances or pollutants. Pollutants can be defined as a chemical or material out of place and present at higher than normal levels, capable of eliciting adverse effects on any organism (Rengel 1999).

The entry of contaminants into an environment can occur through two distinct routes, namely natural environmental processes and human activity. The former means of contaminant entry into an ecosystem originates from either excessive weathering of bedrocks or displacement of certain contaminants from the groundwater or sub-surface layers of the soil. However, this route of contaminant entry is limited in comparison to the human-mediated entry route (Dean et al. 1972). The most common routes of human-mediated entry of contaminants into agricultural and industrial lands are (a) disposal of industrial effluents and sewage sludge, (b) deposition of air-borne industrial wastes, (c) military, mining and land-fill operations, (d) petroleum industry operations, (e) industrial solid waste disposal, and (f) excessive use of agricultural chemicals such as pesticides, herbicides and fertilizers (Saxena et al. 1999; Ross 1994). In general, these environmentally detrimental pollutants can be classified under two major groups: a) hydrocarbon / organic contaminants, and b) inorganic contaminants. Although most organic contaminants

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are introduced into the environment either through industrial or agricultural processes, the inorganic contaminants, especially metal contaminants, may occur naturally in soil or may originate from industrial and mining processes. Irrespective of the contaminant type, substances that are required for industrial or agricultural operations become hazardous contaminants when they build-up in the growth matrix, or are present at toxic levels.

The most common contaminants in the environment are: (a) petroleum hydrocarbon wastes (benzene, toluene, ethylbenzene, xylenes; BTEX), polycyclic aromatic hydrocarbons (PAHs), pentachlorophenol, polychlorinated biphenyls (PCBs), chlorinated aliphatics (trichloroethylene, tetrachloroethylene, 1,1,2,2-tetrachlroethane), (b) ammunition wastes (2,4,6-trinitrotoluene; TNT, RDX), (c) metals (Pb, Cd, Ni, Cu, Co, V, Zn, Mo, Cr, As, Hg, Se), (d) pesticide wastes (atrazine, cyanazine, metolachlor), (e) radionuclides (cesium-137, strontium-90, uranium), and (f) nutrient wastes (ammonium, phosphates, nitrates). The focus of this review will be primarily towards heavy metal contaminants.

Heavy metal contamination

All living organisms (plants and animals) require trace elements for their normal growth and development. Elements such as Cu, Zn, Fe, Mn, Mo, Ni, and Co, through their involvement in various enzymes and other physiologically active molecules, play a significant role in gene expression; biosynthesis of proteins, nucleic acids, growth substances, chlorophyll and secondary metabolites; and carbohydrate and lipid metabolism (Rengel 1999). In addition, these elements are also involved in the structural and functional integrity of various membranes and other cellular components (Rengel 1999). However, these nutrients become toxic when their concentration limit is exceeded. Metal contaminated soils may contain excess amounts of any or all of metals such as Pb, Cd, Ni, Cu, Co, Va, Zn, Mo, Cr, As, Hg, and Se, in varying combinations and concentrations. The problem of heavy metal contamination is further aggravated by the persistence of these metals in the environment. For example, Pb can persist in the environment for 150-5000 years (Friedland 1990), thereby imposing environmental and human / animal health risks, which so far lack an effective and affordable remediation technology (Salt et al. 1995).

Scope of the remediation industry

Mounting pressures due to increasing human population, growing economies and excessive resource-use have all resulted in a cumulative increase in environmental pollution. Concomitant with the increase in contaminated land and water systems, there has been an increase in remediation costs. In 1995, Salt et al. estimated that the clean-up costs of heavy metal contaminated soils alone in the US was US$ 7.1 billion, while metals present in tandem with organic contaminants (as is the case in most contaminated soils) would escalate the costs to US$ 35.4 billion. The US Environmental Protection Agency (Cleaning Up the Nation's Waste Sites: Markets and Technology Trends, EPA 1999) estimates that there are more than 217,000 contaminated sites from past governmental remediation activities, and has estimated a staggering $ 187 billion cost for cleaning up these sites. Environment Canada has estimated that the potential cost for remediating contaminated soils in Canada is $ 6-20 billion. According to OECD (Organization for Economic Co-operation and Development) estimates, the total global market for environmental products and services will grow at an annual rate of 20% to $ 500 billion in 2000.

Impediments to existing clean-up methodologies

There are numerous physical, chemical, and thermal techniques available for remediation of contaminated sites ranging from soil flushing, pneumatic fracturing, solidification/stabilization, vitrification, electrokinetics, chemical reduction / oxidation, soil washing, and excavation, retrieval and off-site disposal. In general, the cost estimates for utilizing most of these technologies, colloquially termed as 'pump-and-treat' and 'dig-and-dump' techniques, have remained high (Table 1). Additionally, these technologies have several disadvantages including ineffective or variable treatment efficiencies and underproduction. Selection and deployment of any remediation technology to alleviate a specific contamination problem is based on several criteria, such as characteristics of the contaminated land, form and concentration of the contaminant, as well as the availability and efficacy of the technology. In most cases, the probable end

Table 1. Cost estimates and time required to effectively employ conventional technologies for soil remediation (from Saxena et al. 1999).

Technology	Cost estimates	Duration
Solidification/ Stabilization	\$50-330/m^3	Medium
Soil flushing	N/A	Medium
Bioremediation	\$30-100/m^3	Long
Electrokinetics	N/A	Medium
Chemical reduction oxidation	\$190-660/m^3	Short
Soil washing	\$120-200/ton	Short
Low temp.thermal desorbtion	\$45-200/ton	Medium
Incineration	\$200-600/ton	Short
Vitrification	\$700/ton	Short
Pneumatic fracturing	\$8-12/ton	Short
Excavation/retrieval disposal	\$270-460/ton	Short
Disposal alone	\$35-60/ton	Short
Landfill disposal alone	\$150-200/ton	Short

N/A - information not available

use of the remediated land also plays a role in the selection of the appropriate technology, as most conventional approaches make the soil infertile and unsuitable for agriculture, by destroying the microenvironment. As one of the emerging, low cost and ecologically friendly technologies phytoremediation addresses these limitations associated with conventional approaches. The use of naturally occurring plants for remediation has therefore gained considerable interest in recent years. Moreover, plant-based remediation is primarily an *in situ* remediation technology and therefore the costs associated with its application are relatively low.

Phytoremediation

Phytoremediation is defined as the effective use of green plants to remove, and render harmless, environmental contaminants in the growth substrate (soil or water) through plant-mediated biological, chemical or physical processes (Cunningham et al. 1995[a]). Plants have evolved a great diversity of genetic adaptations and are equipped with remarkable metabolic absorption capabilities in addition to transport systems that can take up ions selectively from the growth substrate. Uptake of toxic ions occurs in plants primarily through the root system that provides an enormous surface area that absorbs and accumulates the water, nutrients essential for growth, as well as non-essential toxic ions. According to Cunningham and Berti (1993), 'green plants' can be redefined as solar-driven pumping and filtering systems that have measurable loading, degradative and fouling capacity. Within this remarkable 'bio-engineering' mechanism, plant roots act as exploratory, liquid phase extractors that can find, alter and/or translocate elements and compounds against large chemical gradients (Cunningham and Berti 1993).

Pro's of phytoremediation

The use of plants for remediation of contaminated substrates has multifold advantages, such as:

- large scale application, as plants can be grown over large areas,
- growing plants is relatively inexpensive and they provide an aesthetic value to the landscape of the site,
- plant-based remediation is environmentally friendly and ecologically safe,
- candidate plants can have potential economic returns to offset the cost of using the technology,
- plants concentrate the contaminants thereby reducing the amount of hazardous waste,
- certain selected plants can survive and remediate very complex contaminated sites containing a mixture of salt + metals + hydrocarbons, and
- disposal or processing of plants used for remediation would require small scale facilities.

Apart from these direct advantages, plants also provide indirect benefits in the form of:

- increased aeration to soil substrate, enabling microbial degradation of organic contaminants or microbe-assisted plant uptake of metals,
- reduced top soil erosion due to plant stand thereby retaining the soil structure and composition, and
- conserving rhizospheric micro-fauna / flora and maintain a healthy micro-ecosystem.

Con's of phytoremediation

Despite the apparent advantages and cost-effectiveness of plant-based remediation technologies, this approach is clearly not a panacea. There are limitations and various issues to be considered in the development and deployment of small or large-scale phytoremedia-

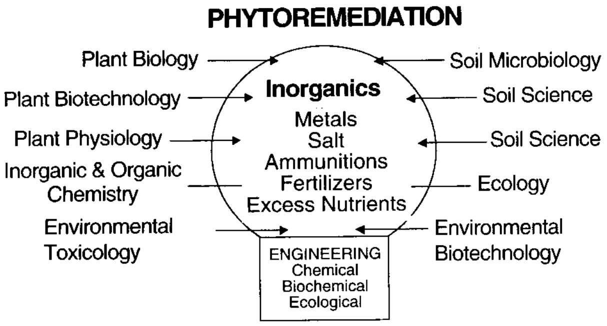

Figure 1. Diagrammatic representation of the multi-disciplinary scope and requirements for developing effective phytoremediation technologies.

tion schemes, such as:

- a comprehensive analysis and understanding of the site is required,
- the selected remediation scheme or technology must be employed properly,
- several science and engineering disciplines are required in adequately assessing and planning phytoremediation of contaminated sites (EPA 1999). It is essential that these are integrated and simultaneously investigated (Figure 1) prior to deployment of this technology,
- the time required for remediation of a contaminated site using phytoremediation can be longer than most conventional technologies,
- the applicability of the technology is limited to sites with surface or shallow sub-surface (rhizospheric) contamination.

Phytoremediation approaches

Phytoremediation technology can be subdivided into different approaches on the basis of the underlying processes and applicability. The subsections of phytoremediation are: a) phytoextraction which refers to using plants to absorb, translocate and sequester toxic contaminants in the shoot tissue (Chaney 1983; Cornish et al. 1995; Kumar et al. 1995); b) rhizofiltration which utilizes the roots to uptake and sequester contaminants from an aqueous substrate (Dushenkov et al. 1995; Dushenkov et al. 1997); c) phytostabilization which involves demobilizing or binding of contaminants into the soil matrix thereby reducing their bioavailability (Cunningham et al. 1995b; Salt et al. 1995); d) phytovolatilization which refers to the plants ability to uptake, transform and volatilize contaminants (Meagher and Rugh 1996; Rugh et al. 1996); and e) phytodegradation / phytostimulation which utilizes the rhizospheric associations between plants and soil microbes to degrade contaminants (Burken and Schnoor 1997; Newman et al. 1997). The use of plants as hydraulic barriers (Gatliff 1994), vegetative caps (Dobson and Moffat 1993), riparian corridors (Licht and Schnoor 1993) can also be included in the overall classification of the phytoremediation approaches. Within the scope of this review, special emphasis will be made on phytoextraction and rhizofiltration approaches.

Phytoextraction

Phytoextraction is the use of metal accumulating plant species to absorb, translocate and sequester toxic ions (metals and others) from soils into the harvestable portions of above-ground biomass. Metal accumulators are plants that can accumulate 10-500 times higher levels of elements than non-accumulator crop species without incurring physiological damage. Reeves (1992) has defined Ni hyperaccumulators as 'plants in which nickel concentration of at least 1,000 μg/g has been recorded in the dry matter of any above ground tissue in at least one specimen growing in its natural habitat' . However, in cases where a plant species accumulates over 10,000 μg/g (1% DW) Ni or Zn, Jaffre et al. (1976) has suggested that the term 'hypernickelophores' or 'hyperzincophores' be used as this ability is qualitatively different than the hyperaccumulators defined by Reeves (1992).

The ability to tolerate and accumulate metals to unusually high concentrations has evolved both independently and together in a number of different plant species (Ernst et al. 1992; Baker and Brooks 1989). Metal hyperaccumulators are taxonomically well represented throughout the plant kingdom (Baker et al. 1989), especially in the Brassicaceae, Euphorbiaceae, Asteraceae, Lamiaceae and Scrophulariaceae families (Baker 1995). For example, *Sebertia acuminata* (a small tree) exudes sap that contains up to 25% Ni by DW (Jaffre et al. 1976). Another example is *Thlaspi caerulescens*, a member of the Brassicaceae family can accumulate up to 4% Zn in its tissue without any visible signs of damage (Brown et al. 1994). Despite a large distribution throughout the plant kingdom, most of the commonly known hyperaccumulators belong to the

Brassicaceae family (Kumar et al. 1995). They probably inherited this characteristic from wild members of the family that are known to thrive in metal rich environments and accumulate metals (Baker 1981, Baker et al. 1994). Some of the plants that have been successfully utilized for phytoextraction are listed in table 2.

Rhizofiltration

Rhizofiltration, on the other hand, is the use of plant roots to absorb, concentrate and precipitate toxic ions from polluted aqueous streams. Unlike phytoextraction, metals are primarily retained in the root system, and not translocated into the shoots. Therefore plants that are used for rhizofiltration may not necessarily be efficient translocators of metals (Salt et al. 1995). An ideal plant for rhizofiltration should have a rapidly growing root system with the ability to remove excessive levels of toxic metals from the solution. Metal bioaccumulation coefficients (the ratio of metal content in plant to the metal content of the growth medium) of roots of some plants are dramatic and can be as high as 60,000 (for example, *Brassica juncea* and *Thlaspi caerulescens*). Some of the plants that have been successfully utilized for rhizofiltration are listed in table 2. In addition plants such as *Agrostis* sp. *Lemna* sp. *Hydrocotyle* sp. *Azolla* sp. *Eichhornia* sp. (Salt et al. 1995) and other wetland plants (Raskin et al. 1994) have been used in rhizofiltration of various metal contaminants.

Factors affecting phytoextraction / rhizofiltration

The utility of phytoextraction / rhizofiltration technology for remediation of metal contaminants is highly dependent on the availability of the metal ions to the roots for uptake which in turn is controlled by chemical, physical and biological processes and their interactions (Ernst 1996). Metals exist in the soil environment in several forms (Salt et al. 1995): a) free metal ions and soluble metal complexes in the soil solution, b) metal ions bound to ion exchangeable sites and specifically adsorbed on to inorganic soil constituents, c) organically bound metals, d) metal precipitates as oxides, carbonates, and hydroxides, e) metals in the structure of silicate minerals. Among those fractions listed above only the first two are readily bioavailable to the plants.

The bioavailability of metals is dependent on several factors. First, it is highly dependent on the soil pH. A decrease in pH (acidification) increases the metal

Table 2. Representative examples of metal accumulator plant species with demonstrated efficacy in phytoextraction and rhizofiltration systems.

Plant Species	Metals	Reference
Phytoextraction		
Alyssum sp.	Ni	Brooks et al. (1979)
		Reeves and Brooks (1983)
Brassica sp.	Pb, Cd, Cu, Ni, Cr (VI), B, Se	Kumar et al. (1995)
		Raskin et al. (1994)
		Salt et al. (1995)
		Banuelos et al. (1997)
Helianthus sp.	Sr	Adler (1996)
Thlapsi sp.	Ni, Zn, Pb	Brown et al. (1994)
		Kumar et al. (1995)
Chenopodium sp.	As	Pierzynski et al. (1994)
Rhizofiltration		
Helianthus sp.	U, ^{137}Cs and ^{90}Sr	Dushenkov et al. (1997)
Brassica sp.	Cd, Ni, Cr (VI), Pb	Salt et al. (1997)
		Dushenkov et al. (1995)
Myriophyllum sp.	Pb, Cd, Cu, Ni, Zn	Wang et al. (1996)
Thlapsi sp.	Zn, Cd	Brown et al. (1995)
Silene sp.	Zn, Cd	Brown et al. (1995)

absorption by plants through a reduction of metal adsorption to soil particles (Brown et al. 1994; Chaney et al. 1995; Huang and Cunningham 1996). Secondly, rhizospheric microbes play a significant role in metal availability. For instance, *Pseudomonas* and *Bacillus* increased Cd accumulation in *Brassica juncea* seedlings (Salt et al. 1995), microorganisms adapted to metal-containing biotopes enhanced Cd, Zn, Hg uptake (Trevors and van Elsas 1997), and plant-mycorrhizal associations enhanced uptake of phosphates as well as metals (Lambert et al. 1976; Killham and Firestone 1983; Joner and Leyval 1997; Marschner 1995).

Thirdly, plant roots also cause changes at the soil-root interface as they release inorganic and organic compounds (root exudates) in the rhizosphere (Kumar et al. 1995). These root exudates affect the number and activity of the microorganisms, the aggregation and stability of the soil particles around the root, and the availability of the elements. Apart from the chelating agents produced by plants, the addition of synthetic chelating agents to contaminated soils was shown to increase substantially the metal solubility in the soil (Salt et al. 1995, Cunningham and Ow 1996). Chelator-assisted phytoextraction is applicable to several metals of interest (Zn, Cd, Ni, Se, As, Cr, U) (reviewed by Salt et al. 1998). For example, the addition of EDTA to a Pb contaminated soil increased the shoot Pb concentration of corn (*Zea mays*) and pea (*Pisum sativum*) (Huang et al. 1997) with a concomitant, more than 1,000-fold, increase in available metal content of the soil solution (Cunningham and Ow 1996). Finally, several other factors such as the redox potential, cation exchange capacity, soil type and texture, rhizospheric conditions, organic matter and clay content, and soil temperature (Kabata-Pendias and Pendias 1992; Webber and Singh 1995; Marschner 1995; Verloo and Eeckhout 1990; Logan and Chaney 1983; Chang et al. 1987) also modulate the bioavailabity of metals (for an indepth review see, Greger 1999).

Scented geranium - a model system

An ideal plant species for phytoremediation, as described earlier, should have one of the following characteristic combinations (Friedland 1990): a) a low biomass plant with a very high metal accumulation capacity, or b) a high biomass plant with enhanced metal uptake potential. In addition to these characteristics, versatility of the candidate plant to tolerate and at the same time accumulate multiple metal contaminants and/or metal-organic mixtures would be an asset. Interestingly, most of the metal hyperaccumulating wild plant species (as defined by Reeves 1992) are relatively small in size and have slow growth rates, thereby limiting their potential in phytoextraction (Salt et al. 1995). This further emphasizes the need for further research to be focused on identification and characterization of metal accumulators in natural habitats as well as a thorough survey of domesticated plant species for selecting potential candidate plants.

One such domesticated species has so far shown considerable potential as a candidate plant in both greenhouse and field trials. A common group of horticultural plants, namely scented geraniums (*Pelargonium* sp.), were found to possess the potential to remediate metal contaminated soils and aqueous systems (KrishnaRaj et al. Method of using *Pelargonium* sp. as hyperaccumulators for remediating contaminated soil, PCT/CA98/ 01027 pending; US 09/185,797 pending). The plants belonging to the *Pelargonium* sp. satisfy all of the prerequisites of a candidate plant species because of their potential to: a) tolerate high concentrations of multi-metal contamination in the growth medium, b) accumulate significant levels of metals in their shoot and root biomass, c) tolerate total petroleum hydrocarbons (up to 30,000 ppm TPH) in the growth medium, and d) tolerate and accumulate salt (NaCl) in the biomass when present alone or in tandem with metal+hydrocarbon contaminants. In addition, significant economic advantages such as, ease in planting and harvesting through conventional farm machinery (seedling transplanters and tuber harvesters), potential economic returns (extraction of value-added essential oils such as citronellol and geranioll) from harvested biomass would offset the cost of deploying the technology. The superior tolerance and metal accumulation potential of scented geranium plants has been extensively investigated.

Metal tolerance of scented geraniums

Metal stress elicits a cascade of phytotoxicity symptoms in plants, such as alterations in growth (Moya et

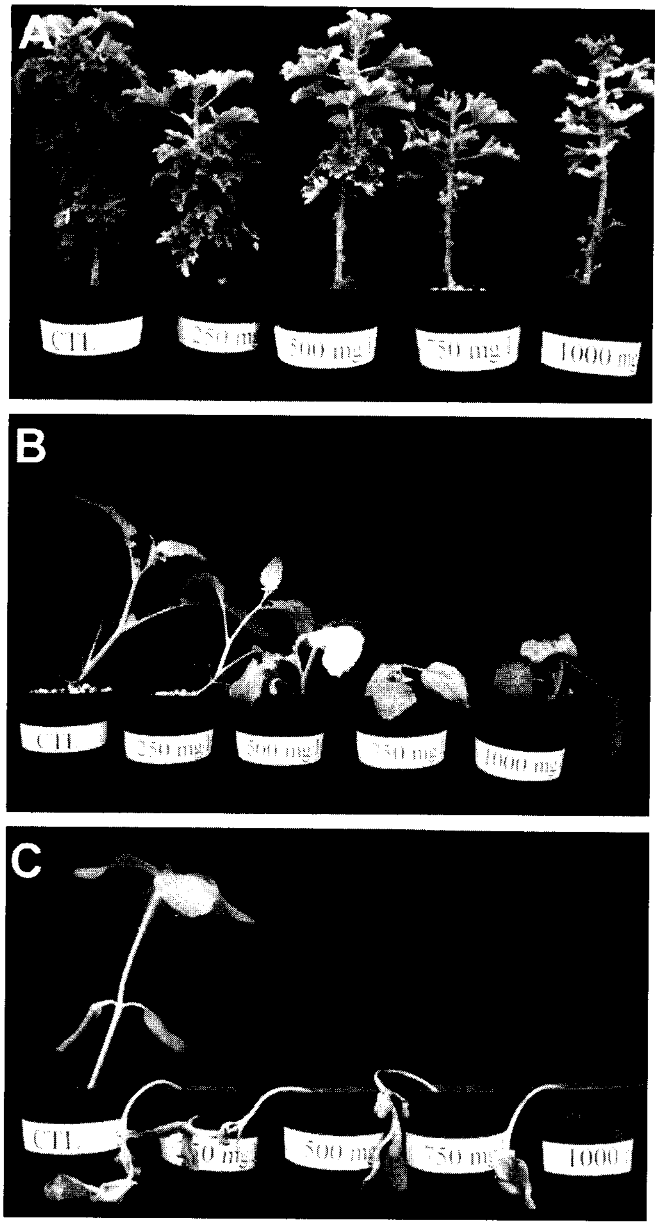

Figure 2. Effect of daily cadmium treatment [0 (CTL), 250, 500, 750 and 1,000 mg/L Cd(NO3)2 · 4H2O] on expression of morpho-phytotoxicity symptoms in A. Pelargonium sp. 'Frensham' (after 14 days), B. Brassica juncea (after 7 days) and C. Helianthus annuus (after 5 days), grown on artificial soil mix (Perlite). CTL, control

al. 1993), perturbations in photosynthesis (Greger and Ogren 1991; Ferreti et al. 1993), carbohydrate metabolism (Malik et al. 1992), water relations (Poschenrieder et al. 1989), mineral nutrition (Rubio et al. 1994) and eventually death (Ernst et al. 1992). Chlorophyll *a* fluorescence kinetics, a non-destructive indicator of the efficiency of the photosynthetic apparatus, was utilized as a quantitative marker to assess the tolerance of scented geraniums to metal (Pb, Cd and Ni) stress. KrishnaRaj et al. (2000) compared the tolerance of *Pelargonium* sp. 'Frensham' with two well-established metal accumulators, *Brassica juncea* and *Helianthus annuus* (Figure 2). In this study, lead exposure did not significantly affect the efficiency of photosystem II (PS II) activity or the number and size of the photosynthetic reaction centers in scented geraniums. However, the PS II activity in *Brassica* sp. and *Helianthus* sp. was significantly inhibited, potentially due to irreversible damage to the photosynthetic apparatus. Consistent with these findings, Dan et al. (2000a) found that cadmium (up to 1,000 mg L^{-1}) or nickel (up to 1,000 mg L^{-1}) did not significantly affect the efficiency of the photosynthetic apparatus. Dan et al. (2000a) have suggested that the metal tolerance in scented geraniums arose from a) maintenance of optimal PS II activity, required for normal plant metabolism and physiological functions, and overcoming metal ion mediated stresses, and b) restriction of damage to the photosynthetic reaction centers by accumulating metal ions.

Metal accumulation/sequestration in scented geraniums

Continued growth and survival of plants in an environment containing various metal contaminants can be either due to avoidance (exclusion of metals from the biomass) or tolerance (uptake of metals in to biomass and detoxification). Although, domestication through breeding and directed selection over the decades has resulted in a majority of plants with the former survival mechanism, plants with enhanced contaminant uptake and detoxification mechanisms have been the focus of phytoremediation research. KrishnaRaj et al. (2000) and Dan et al. (2000b) have successfully demonstrated the metal accumulation potential of scented geraniums. In greenhouse pot trials, *Pelargonium* sp. 'Frensham' were found to accumulate in excess of 750 mg of Cd or 1,200 mg Ni or 3000 mg of Pb per kg DW of shoot and 27, 000 mg of Cd or 21,100 mg of Ni or 60,000 mg of Pb per kg DW of root tissue within 14 days of exposure to 0-1,000 mg/L $Cd(NO_3)_2 \cdot 4H_2O$ or 0-1,000 mg/L $Ni(NO_3)_2 \cdot 6H_2O$ or 0-2,500 mg/L $Pb(NO_3)_2 \cdot 6H_2O$, respectively. Additionally, the metal distribution pattern in the plant biomass indicated that the metal accumulation process was a metabolically controlled mechanism, which varied with the specific metal studied.

Although the focus of this review is not at elucidating the mechanisms of metal ion entry into the root cells or its translocation to the shoot (see reviews by Dan et al. 1998; Greger 1999), it is worthwhile to note that the scented geraniums provide us with an ideal model system to study these processes, due to the multi-metal tolerance and accumulation mechanisms. The cadmium accumulation in the biomass of scented geraniums was found to be directly proportional to the external Cd concentration, similar to most known metal accumulators (Greger and Lindberg 1986). Dan et al. (2000b) have suggested that the Cd uptake in scented geraniums was likely through both active and passive transport mechanisms, as the toxicity threshold was not exceeded even at the elevated levels of external Cd and the metabolic control of Cd uptake was not lost. In contrast, Cutter and Raius (1974) found that a large fraction of Cd taken up by barley was through exchange absorption and through diffusion coupled with sequestration, without any concomitant active metabolic uptake. However, in the case of Ni accumulation in scented geraniums, the root Ni concentration did not increase with the applied metal concentration, indicative of a predominantly active uptake of this metal (Dan et al. 2000b).

Metal accumulation in root tissue can be accomplished either through deposition of the metal ions along the cell wall and/or inside the cell in the vacuoles. The sequestration of specific metal ions or metal-chelate complexes in the root cells is highly dependent on the metal ion in question. For example, Pb is generally found to be associated with cell walls outside the plasmalemma in the form of Pb precipitates and Pb crystals (Malone et al. 1974). Similar to Pb, large concentration of Cd (Hardiman et al. 1984) have also been associated with cell walls, while Zn was primarily sequestered in the vacuole (Brookes et al. 1981). In the case of scented geraniums, KrishnaRaj et al. (2000), using transmission electron microscopy coupled with an energy-dispersive X-ray microanalyzer, found that lead was sequestered in the apoplasm, cytoplasm, vacuole and as distinct globules on cell membranes and the cell wall. They suggested that hyperaccumulation of lead in the root tissue of scented geraniums was in part due to the formation of electron opaque, metal-lignin complexes which were sequestered on the cell walls (consistent with the increased PAL activity associated with the lignification process). The lead accumulation in scented geraniums was also found to follow a decreasing gradient starting from the epidermis towards the central axis of the root (KrishnaRaj et al. 2000) indicative of the presence of both passive and active metal uptake and translocation mechanisms in this plant species.

Metal accumulation in shoots of several accumulator plants is attributed to active detoxification mechanism(s) (Verkleij and Schat 1980) such as the production of intercellular compounds, metal compartmentalization patterns or an increased cellular metabolism. In general, the mechanisms of metal translocation seem to be very similar to those for essential nutrients such as Fe or Ca; in as much as the transport from the root to the shoot takes place principally through the xylem. Several types of compounds have been proposed to be involved in metal absorption and translocation in accumulator plant species. In the case of scented geraniums, the difference in accumulation of Cd and Ni is probably dependent on the form in which these metals are translocated to the shoots (Dan et al. 2000b). It is likely that, Ni is transported in association with citrate (Lee et al. 1977), or as a nickel-peptide or a nickel-histidine complex (Krammer et al. 1996), while Cd is transported as a cadmium-citrate complex (Senden et al. 1992).

Salt tolerance and accumulation in scented geraniums

Garnett et al. (2000) assessed the ability of scented geraniums to tolerate salt stress (NaCl) using a hydroponic rhizofiltration system. In these experiments, the scented geraniums did not exhibit any morpho-phytotoxicity symptoms when grown on hydroponic solutions containing up to 100 mM NaCl. Consistent with the metal tolerance, assessed using chlorophyll *a* fluorescence parameters, scented geraniums were found to tolerate NaCl stress by limiting damage to the photosynthetic apparatus (Garnett, KrishnaRaj, Dixon, Saxena, unpublished data). In contrast to the metal accumulation patterns observed by Dan et al. (2000b) and KrishnaRaj et al. (2000), salt accumulated initially (up to 10 days) in the root tissue, followed by significant translocation to the shoot tissue (Garnett et al. 2000). Although the accumulation pattern was season-dependent, scented geraniums accumulated in excess of 37,000 mg Na per kg DW of shoot and 26,000 mg Na per kg DW of root tissue. Garnett et al. (2000) suggested that the translocation of sodium from the roots to the

Table 3. Soil metal contents and metal accumulation in *Pelargonium* sp. 'Frensham' exposed to land-farming soils containing mixture of multi-metal contaminants and 3% total petroleum hydrocarbons for 14 d, in greenhouse propagation tray experiments. Values are means of 3 replicate plant or soil samples in one experiment and the experiment was repeated once.

Element	Soil metal concentration prior to phytoremediation (mg/kg soil)	Shoot metal content (mg/kg DW)	Root metal content (mg/kg DW)	Percent decrease in soil metal concentration after phytoremediation
Boron	13.0	110.5	38.0	0.0
Cobalt	12.0	<1.0	7.5	0.0
Copper	180.0	33.5	105.0	5.6
Cadmium	1.1	0.9	0.3	23.8
Lead	115.0	91.6	35.0	4.3
Molybdenum	14.5	6.6	10.2	10.3
Nickel	73.5	5.9	37.7	0.7
Vanadium	115.0	3.3	62.0	4.3
Zinc	467.5	80.0	260.0	12.3

shoots was probably modulated by a flux of primary metabolites (GABA, proline, asparagine, glutamine and alanine) associated with the nitrogen assimilation, which increased in the root tissue up to day 10 followed by accumulation in the shoot tissue.

Greenhouse contaminated soil trial

The efficacy of scented geraniums to remediate contaminated soils (containing low levels of multi-metal contamination in addition to 3 % total petroleum hydrocarbons [TPH]) was assessed using land-farming soils in greenhouse pot trials (KrishnaRaj, Dan and Saxena, unpublished data). The efficacy of scented geranium (30 d old cuttings) was compared with 30 d old seedlings of Indian mustard grown in greenhouse propagation trays containing soil sampled from a petroleum industry land-farming site and one control tray containing Promix (greenhouse soil mix). Scented geraniums accumulated significant levels of Cu, Cd, Pb, Mo, Ni, V and Zn from the land-farm soil (Table 3), while Indian mustard succumbed to the metal + hydrocarbon stress. Following the 14 d remediation cycle with scented geraniums, the individual metal contents in the soil decreased to varying degrees (ranging from 24% reduction in Cd levels to 0.7% reduction in Ni). Although, significant uptake of B and Co was observed in scented geraniums, the soil B and Co contents remained unaltered. Also, certain replicate soil samples showed increased metal content following treatment with scented geraniums. The increase in soil metal levels, following treatment with scented geraniums, could be attributed in part to either the non-homogeneity of the soil sample or the increased metal mobilization in the soil matrix fostered by scented geranium root exudates. These observations confirm the metal tolerance, accumulation characteristics of scented geraniums and its utility as an ideal plant for remediation of mixed contaminant soils (TPH-laced, multi-metal contaminated soils). The uptake of metal contaminants from contaminated soils in greenhouse pot experiments has been shown to be positively correlated and reproducible in the field (Huang et al. 1997). Although, it would be premature to speculate on the efficacy of scented geraniums in remediating metal contaminated soils without field trials (currently underway, Spring-Summer 2000). Based on extrapolation of greenhouse trial results, it is likely that up to 15 plantings (within 5-7 years, @ 2-3 plantings/ year) of scented geraniums will be required to effectively remediate these land-farm soils.

Fate of contaminants in scented geraniums

The contaminant uptake and accumulation pattern in scented geraniums varied significantly and was found to be highly dependent on the contaminant type (Figure 3). In the case of metal contaminants, a significant proportion of the metal taken up by the roots was sequestered within the root biomass as metal complexes on the cell walls and cell membranes, while a lower pro-

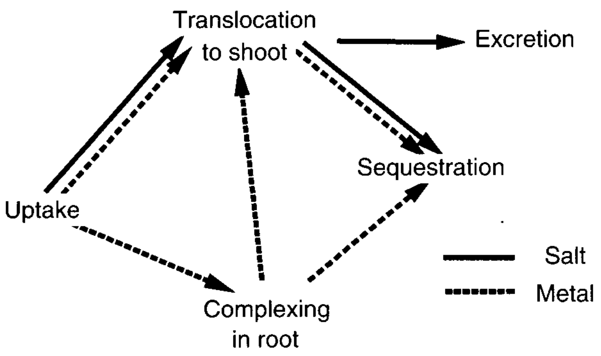

Figure 3. Potential fate of contaminants (metals such as Pb, Cd and Ni) and salt (NaCl) during phytoextraction / rhizofiltration in scented geraniums.

portion was translocated to the shoot tissue (KrishnaRaj et al. 2000). However, in the case of salt, the uptake and accumulation of Na ions in the root occurred in the earlier days of exposure followed by increased translocation to the shoot, probably once the root Na content exceeded the threshold (Garnett et al. 2000). Additionally, the translocated salt was also excreted from the leaf/petioles of scented geraniums, indicative of a functional exclusion mechanism for salt away from the sensitive metabolic sites. These findings point to the existence of more than one functional tolerance and contaminant-detoxification mechanism in scented geraniums. It is also evident that all of these mechanisms operate either individually or in tandem, for alleviating the imposed metal and/or salt stress depending on the conditions. This unique feature of scented geraniums renders this plant the ideal choice for remediating sites with complex contamination (metals + salt + hydrocarbons). The utility of this plant species can be further enhanced through the use of metal-chelating agents or by incorporating gene(s) that encode for metal binding proteins and polypeptides. Such approaches would result in 'super' -accumulator plants, with far-reaching applications for the phytoremediation industry. In addition, the unique multi-metal tolerance characteristics and high amenability to genetic transformation (KrishnaRaj et al. 1997) render scented geraniums as an ideal system for investigating the biochemical and molecular basis of metal tolerance in plants.

Conclusions

Environmental pollution is a complex global problem. In the past, national and global drives towards economic growth have contributed significantly to the deterioration of the environment and quality of life in terms of human and livestock health. There is considerable public support for the idea that our current ways of life are generating problems for the future and that economic activity must be held within environmental limits. Economic growth is only one component of the quality of life and would have little meaning in a polluted environment. The emerging field of phytoremediation offers us a low-cost, ecologically safe alternative to conventional remediation technologies for controlling this persistent global problem of environmental pollution. However the success of the phytoremediation technology primarily depends on the selection of the ideal plant species. The model system, *Pelargonium* sp. described in this review, is one such plant species with demonstrated efficacy in remediating metal and salt contaminated soils. We conclude that further identification and characterization of metal accumulators (plants in natural habitats or domesticated plant species) and enhancing the metal accumulation potential of identified species, (such as scented geraniums) through biotechnological means, will facilitate the utility of this technology on a commercial scale.

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