Environmental cleanup using plants: biotechnological advances and ecological considerations

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Plants and their associated microbes can be used in the cleanup and prevention of environmental pollution. This relatively new and growing technology uses natural processes to break down, stabilize, or accumulate pollutants. Knowledge of the biochemical processes involved may lead to the development of more efficient plants and better management practices. One approach for improving the efficiency of phytoremediation includes developing transgenic plants. Here, we give an overview of phytoremediation methods and their associated biological processes, and discuss approaches that have been used successfully to breed transgenic plants with advanced phytoremediation properties. Much is still unknown about the ecological implications of phytoremediation, especially when using transgenic plants. Phytoremediation-related processes can change the location or chemical makeup of contaminants; the question is how those processes will affect the interactions among organisms in the ecosystem, and how transgenic plants might influence these relationships. Continued multidisciplinary studies will result in a better understanding of the ecological interactions that contribute to phytoremediation, the effects of phytoremediation on ecological relationships, and the movement of pollutants through ecosystems.

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Our planet is becoming increasingly polluted with inorganic and organic compounds, primarily as a result of human activities. While inorganic pollutants occur as natural elements in the Earth's crust and atmosphere, human activities such as industry, mining, motorized traffic, agriculture, logging, and military actions promote their release and concentration in the environment, leading to toxicity (Nriagu 1979). Organic pollutants in the environment are mostly man-made and xenobiotic (ie not normally produced or expected to be present in organisms). Many of them are toxic and/or carcinogenic. Sources of organic pollutants in the environment include accidental releases (eg fuels, solvents), industrial activities (eg chemical, petrochemical), agriculture (eg pesticides, herbicides), and military activities (eg explosives, chemical weapons), among others. Moreover, polluted sites often contain a mixture of both organic and inorganic pollutants (Ensley 2000). Currently $6–8 billion a year is spent on environmental cleanup in the US, and $25–50 billion per year worldwide (Glass 1999; Tsao 2003). Most remediation activity still makes use of conventional methods such as excavation and reburial, capping, and soil washing and burning. However, newly emerging biological cleanup methods, such as phytoremediation, are often simpler in design and cheaper to implement.

In a nutshell:

- Phytoremediation, the use of plants and their associated microbes, is an emerging technology in the cleanup and prevention of environmental pollution
- We present an overview of what is known about phytoremediation processes and the transgenic approaches used to breed plants with better phytoremediation properties
- The ecological interactions influencing phytoremediation and the implications of the use of phytoremediation, including transgenics, are discussed
- Better knowledge of the ecological implications of these techniques will help improve risk assessment during remediation design, as well as minimizing the associated risks

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Phytoremediation incorporates a range of technologies that use plants to remove, reduce, degrade, or immobilize environmental pollutants from soil and water, thus restoring contaminated sites to a relatively clean, non-toxic environment. Phytoremediation depends on naturally occurring processes, in which plants detoxify inorganic and organic pollutants, via degradation, sequestration, or transformation. The different uses of plants and their associated microbes for environmental cleanup are summarized below (for reviews, see also Salt *et al.* 1998; Meagher 2000; Pilon-Smits 2005).

Phytoextraction is the removal of pollutants by the roots of plants, followed by translocation to aboveground plant tissues, which are subsequently harvested. Continuous phytoextraction uses plants that accumulate high levels of pollutants over their entire lifetime. Induced phytoextraction enhances pollutant accumulation towards the end of the plant's lifetime, when they attain their maxi-

Courtesy of G Bañuelos, USDA-ARS

Figure 1. *Canola, a cultivar of rapeseed (*Brassica napus*), being used to remediate seleniferous (selenium-contaminated) soils in the San Joaquin Valley, CA. Canola holds great potential as a phytoremediator of sites with high concentrations of selenium because of its flexibility, as it is able to remove the element through both phytoextraction (ie accumulation in harvestable tissue) and phytovolatilization (ie emission of dimethylselenide, a volatile form of selenium).*

mal biomass, by adding chelators to the soil that reversibly bind the pollutant (usually a metal), releasing it from the soil and making it available for plant uptake. Phytoextraction is especially useful when dealing with toxic pollutants that cannot be biodegraded, such as metals, metalloids, and radionuclides. One category of plants that shows potential for phytoextraction, either as a gene source or for direct use, are the so-called hyperaccumulators, plants that accumulate toxic elements to levels that are at least 100-fold higher than non-accumulator species (Baker and Brooks 1989; Peer *et al.* 2005). Hyperaccumulator plants tend to grow relatively slowly, which limits their usefulness for phytoremediation. Nevertheless, their growth rate may be improved through selective breeding (Chaney *et al.* 2000), and the transfer of metal hyperaccumulation genes to high-biomass, fast-growing species may also help to circumvent the problem (LeDuc *et al.* 2004).

Phytodegradation involves the partial or complete degradation of contaminants by internal or secreted plant enzymes. As in phytodegradation, *phytostimulation* also involves enzymatic breakdown, but through microbial activity. Plants can stimulate microbial biodegradation in several ways. Since most organic pollutants can be broken down enzymatically, phytodegradation and phytostimulation are particularly effective for this class of pollutants.

Phytovolatilization depends on the uptake of contaminants by plant roots, followed by their release as volatile chemicals by either the root or the shoot. While some pollutants are volatile to begin with, and are simply dispersed unaffected into the atmosphere, others, such as inorganic selenium, can be transformed enzymatically into volatile forms during the process of plant uptake and release (Figure 1).

Rhizofiltration uses plant roots to filter contaminants directly out of waste streams, in either a hydroponic or a constructed wetland setting. Rhizofiltration is also suitable for inorganics, as the plant material can be replaced periodically.

Erosion and leaching often mobilize soil contaminants, resulting in additional aerial or waterborne pollution. *Phytostabilization* through accumulation by plant roots or precipitation in the soil by root exudates immobilizes and reduces the availability of soil pollutants. As an added benefit, plants growing on polluted sites also stabilize the soil and serve as groundcover, thereby reducing both wind and water erosion.

Inorganics are generally dealt with by phytoextraction and/or phytostabilization, while organics are most commonly treated by phytodegradation and phytostimulation. Phytoremediation in general may involve a combination of the technologies described above. At the same time, pollutants may be stabilized, degraded, sequestered, and/or volatilized. In fact, an advantage of phytoremediation over conventional remediation is the ability to remove a variety of organic and inorganic contaminants from a site concurrently. The complete phytoremediation of contaminated soils to fully functioning soils is called *phytorestoration*.

Phytoremediation is being used successfully to deal with a wide range of solid, liquid, and gaseous substrates (Raskin and Ensley 2000; McCutcheon and Schnoor 2003). Appropriate locations for phytoremediation include military sites (trinitrotoluene [TNT], metals, organics); agricultural fields (herbicides, pesticides, metals, selenium); industrial sites (organics, metals, arsenic); mine tailings (metals); wood treatment sites (polycyclic aromatic hydrocarbons); sewage and municipal wastewater (nutrients, metals); agricultural runoff/drainage water (fertilizer nutrients, metals, arsenic, selenium, boron, organic pesticides, and herbicides); industrial wastewater (metals, selenium); coal pile runoff (metals); landfill leachate; mine drainage (metals); groundwater plumes (organics, metals); and outdoor and indoor air (NO_x, SO_2, ozone, CO_2, nerve gases, dust or soot particles, and halogenated volatile hydrocarbons).

Depending on the fate of the pollutant, vegetation used in phytoremediation may require further processing. In the case of phytoextraction, typically used for inorganics such as metals, the plant material can be further concentrated by composting or ashing (producing bioenergy), and either disposed of in a landfill or used to recycle the element. The latter process, termed *phytomining*, is currently being used for nickel (Chaney *et al.* 2000). In cases involving phy-

Table 1. Cost comparison of two popular phytoremediation technologies (shown in bold) with alternative remediation methods for soils contaminated with organics (Schnoor 1997) or inorganics (Glass 1999)

Type of treatment	*Range of costs ($/ton soil)*
Organics	
Rhizosphere degradation	**$10–$35**
In situ bioremediation	$50–$150
Soil washing	$80–$200
Soil venting	$20–$220
Solidification/stabilization	$240–$340
Solvent extraction	$360–$440
Incineration	$200–$1500
Inorganics	
Phytoextraction	**$25–$100**
Soil washing	$50–$150
Solidification/stabilization	$75–$205
Soil flushing	$75–$210
Electrokinetics	$50–$300
Acid leaching/extraction	$150–$400
Landfilling	$100–$500
Vitrification	$40–$600

todegradation or phytostimulation, no further processing may be needed, depending on the toxicity of the end products. However, this must be evaluated on a case by case basis. In instances of volatilization, the volatile product is dispersed and diluted, decreasing its toxicity. This makes volatilization an attractive remediation process, as it is not believed to pose a significant health or environmental hazard, even when used for the absorption of mercury (Meagher *et al.* 2000).

Advantages and limitations of phytoremediation

A big advantage of phytoremediation over more traditional remediation methods is that in most cases it is less expensive. Depending on the pollutant, substrate, and alternative remediation methods available, phytoremediation is typically 2–10-fold cheaper than conventional remediation methods (Table 1; Schnoor 1997; Glass 1999). It is also less invasive and more aesthetically pleasing compared to excavation and removal, chemical stabilization, or soil washing or incineration (EPA 1998, 1999). The ability to use plants in various capacities for different synergistic processes, resulting not only in environmental cleanup but also in ecosystem restoration, is the biggest advantage. Tailoring the phytoremediation technique to the specifics of a polluted environment will become more feasible as more information becomes available; for example, in the future it may be possible to select a combination of plant species with different remediation capabilities to clean up sites containing a mix of contaminants. Preferentially, native plant species will be used in order to promote ecosystem restoration during the cleanup process.

A limitation of phytoremediation is that the plant roots have to be able to reach the pollutant and act on it. Thus, the soil characteristics, toxicity level, and climate have to be amenable to plant growth, while the pollutant must not only be within physical reach of the roots, it must be bioavailable for absorption as well. Phytoremediation also usually takes longer than conventional methods. Flow-through filtration systems and plant degradation of pollutants generally work fairly fast (from days for filtration up to several years for degradation), but soil cleanup via plant accumulation may takes years to decades. Some of these limitations may be circumvented by deep planting and by amending the soil with substances that either make it more amenable to plant growth (eg lime, compost), or that make the pollutant more bioavailable (eg chelators, surfactants).

Mechanical remediation technologies and phytoremediation can be used in conjunction. Pollutant distribution and concentration are heterogeneous at many sites, so the most efficient remediation solution may be a combination of different approaches, eg excavation of the most contaminated spots, followed by phytoremediation.

Developing transgenic plants for phytoremediation

Transgenic plants are genetically modified organisms. In genetic engineering, plants are induced to take up a piece of DNA containing one or a few genes originating from either the same plant species or from any different species, including bacteria or animals. The foreign piece of DNA is usually integrated into the nuclear genome, but can also be engineered into the genome of the chloroplast. Foreign DNA may cause an existing enzymatic activity to become up-regulated (overexpression) or down-regulated (knockout/knockdown), or may introduce an entirely new enzymatic activity altogether. The expression of the introduced gene can be regulated by using different promoters. The gene product, a protein, may be present at all times, in all tissues (constitutive expression), or only in certain tissues (eg only in roots) or at certain times (eg only in the presence of light or a chemical inducer). Moreover, using different targeting sequences, which function as "address labels", the protein may be directed to different cellular compartments, such as the chloroplast, the vacuole, or the cell wall. In addition to the gene of interest, a marker gene is usually included in the gene construct so that transgenics can be selected for after the transformation event. Usually these marker genes confer herbicide or antibiotic resistance. The introduced genes integrate into the host DNA and are inherited by the offspring like any other gene.

In the context of phytoremediation, it is desirable to engineer high-biomass producing, fast-growing plants with an enhanced capacity to tolerate pollutants. In addition, if a pollutant is remediated via accumulation, as is often the case for inorganics, transgenics may be engineered to possess improved pollutant uptake and root–shoot translocation abilities. If the pollutant is remediated by degradation, as organics often are, enzymes that facilitate degradation in either the plant tissue or the rhizosphere (the region just outside of the root) may be overexpressed. In cases where pollutants are volatilized, enzymes involved in the volatilization process may be overexpressed.

If a transgenic approach is to be used to breed plants with superior phytoremediation properties, it is necessary to understand the underlying mechanisms involved. Once potential rate-limiting steps have been identified by means of physiological and biochemical experiments, the specific membrane transporters or enzymes responsible can be singled out for overexpression. If the genes encoding these proteins are available from any organism, they can be introduced into the plant and the transgenics can be compared with the wild type (non-transgenic) with respect to pollutant remediation. This will provide information about fundamental plant biology and may lead to the development of plants that can be used for environmental cleanup.

A great deal of research has been carried out to investigate mechanisms involved in plant uptake of inorganic and organic pollutants and their fate in the plant (for reviews see Meagher 2000; Burken 2003). Generally, inorganics are taken up by transporters for essential elements, advertently if they are indeed essential, or inadvertently if they are chemically similar to essential elements. Once inside the plant they may be detoxified by chelation and by compartmentation in a safe place such as the vacuole. Organics can move passively across plant membranes if they have the right degree of hydrophobicity, corresponding to a log K_{ow} (octanol:water partition coefficient) of 0.5–3. More hydrophilic organics cannot pass the hydrophobic interior of membranes passively, and there is usually no suitable transporter if they are foreign to the plant. More hydrophobic organics tend to stick to soil particles, thereby reducing their bioavailability, or they become stuck inside root membranes and are prevented from moving into the cell's interior. Organic pollutants that do make it into the plant can be detoxified by enzymatic degradation. They may also be stored in the vacuole or cell wall, after enzymatic modification and conjugation to glutathione or glucose, the latter referred to as the "green liver model" (Burken 2003).

■ Advances in breeding transgenics for environmental cleanup

Plants have already been engineered to better tolerate and/or accumulate various organic and inorganic pollutants, making use of our current knowledge of plant mechanisms involved in pollutant tolerance and accumulation (for reviews see Meagher 2000; Kramer and Chardonnens 2001; Pilon-Smits 2005).

Generally, plant species that are easy to transform and have a short generation time, such as *Arabidopsis*, tobacco (*Nicotiana tabacum*), or Indian mustard (*Brassica juncea*), are used for testing transgenic approaches in initial lab and greenhouse experiments. *B juncea* is also a popular species for phytoremediation, as it is an efficient accumulator of inorganics, grows quickly, and attains a high biomass. Hybrid poplar (*Populus* sp), a perennial, fast-growing, and environmentally tolerant tree with a high transpiration rate which aids in the translocation of pollutants to the shoot, is another favored species for phytoremediation. Another tree species that is being tested in remediation studies is yellow poplar (*Liriodendron* sp). Both *Populus* and *Liriodendron* can be genetically engineered. Genetic engineering programs for phytoremediation are also underway for the development of transgenic wetland species, such as *Spartina* spp, reeds, and *Typha* spp (Czako *et al.* 2005).

A variety of genes have been used in genetic engineering for phytoremediation. For instance, bacterial enzymes involved in degradation of the explosive TNT were expressed in plants, resulting in enhanced plant TNT tolerance and degradation (French *et al.* 1999). Furthermore, expression of a mammalian cytochrome P450 in plants resulted in enhanced ability to metabolize the organic solvent trichloroethylene (Doty *et al.* 2000). Various transgenic plants were created with augmented inorganic pollutant tolerance and accumulation properties, either by overexpression of membrane transporter proteins (Hirshi *et al.* 2000; Song *et al.* 2003; Van der Zaal *et al.* 1990) or by overproduction of chelator molecules (Goto *et al.* 1999; Zhu *et al.* 1999a,b; Dhankher *et al.* 2002). Plant volatilization has also been enhanced through genetic engineering. Overexpression of two key enzymes – cystathionine-gamma-synthase and selenocysteine methyltransferase – was shown to promote the con-

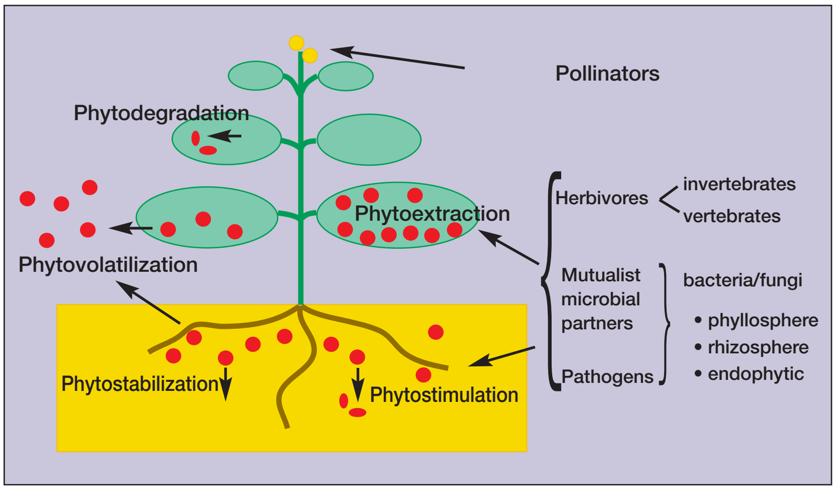

Figure 2. *Schematic overview of phytoremediation methods. Shown on the right are some ecological partners of the plants that may influence phytoremediation efficiency and may, in turn, be affected by the phytoremediation process.*

version of selenocysteine to volatile selenium (van Huysen *et al.* 2003; LeDuc *et al.* 2004). Volatilization of mercury by plants was achieved through the introduction of a bacterial mercury reductase (MerA); the resulting plants volatilized elemental mercury and were significantly more mercury-tolerant (Rugh *et al.* 1996).

While most of the testing of transgenics for phytoremediation has been done in the laboratory and greenhouse, a few studies have been undertaken using polluted soil taken from contaminated sites, and one study has been done in the field directly. These studies have confirmed results obtained in laboratory experiments. Transgenics engineered to have higher levels of metal chelators showed enhanced cadmium and zinc accumulation in greenhouse experiments using polluted soil (Bennett *et al.* 2003). Also, transgenic plants engineered to have enhanced sulfate/selenate reduction showed 5-fold higher selenium accumulation in the field (Bañuelos *et al.* 2005). A field experiment testing mercury-volatilizing poplar trees is presently underway (D Glass pers comm).

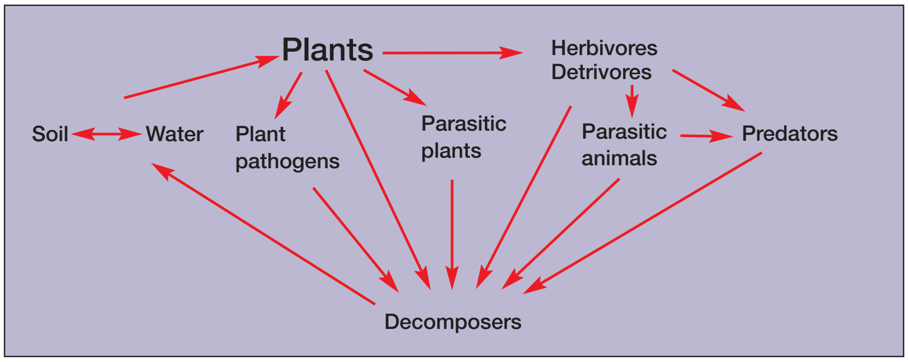

Figure 3. *Schematic overview of possible movement of pollutants through the ecosystem. The paths taken and the relative fluxes for each path will depend on the pollutant, the plant species involved, and local conditions.*

■ Ecological considerations

Many ecological issues need to be evaluated when developing a remediation strategy for a polluted site. In particular, one has to consider how the phytoremediation efforts might affect local ecological relationships. As described above and shown in Figure 2, phytoremediation-related processes can change the location or chemical makeup of contaminants in the polluted area. The question is, how do those processes affect the ecological interactions among organisms in the ecosystem? The choice of plant species for remediation will, of course, greatly influence which ecological partners and interactions will be present at the site, and consequently the fate of the pollutant. The direct ecological partners of phytoremediator plants include bacteria, fungi, animals, and other plants, all occurring inside, on, or in the vicinity of the roots and shoots of the phytoremediator plants (Figure 2). These partners may be affected positively or negatively by the ongoing phytoremediation process. If the plants stabilize or degrade the pollutant, thereby limiting its bioavailability and concentration, the phytoremediation process will probably benefit other organisms in the area. If, on the other hand, the plants accumulate the pollutant or its degradation products in their tissues, this may adversely affect microorganisms that live on or inside the plant (Angle and Heckman 1986), as well as root and shoot herbivores, and pollinators. Volatilization of a pollutant will simultaneously dilute and disperse the pollutant, which may affect ecosystems both on and off the site.

In addition to the direct ecological partners of the phytoremediator plants, the phytoremediation processes may also affect other trophic levels. If a pollutant is accumulated by the plant, this may facilitate its entry into the food chain, as depicted in Figure 3. Conversely, these ecological partners may affect the remediation process positively or negatively, by interacting with the pollutant directly or with the plants. Herbivores or pathogens may hamper plant growth and thus the phytoremediation efficiency. On the other hand, rhizosphere or endophytic microorganisms may make pollutants more bioavailable for plant uptake, or may assist in the biodegradation process. While it is known that plant–microbe consortia often work together in remediation of organic pollutants (Olson *et al.* 2001; Barac *et al.* 2004; Van Aken *et al.* 2004; Taghavi *et al.* 2005), much still remains to be discovered about the nature of the interactions and the molecular mechanisms involved (eg signal molecules, genes induced).

■ Ecological studies

Relatively little is known at this point about the ecological effects of the use of plants in phytoremediation. The study of the ecology of metal hyperaccumulator plants may give some insight into the effects toxic elements in plant tissues may have on the plant's ecological partners. Nickel or zinc hyperaccumulating plants have been shown to be protected against herbivory, due to the toxicity of the metal in their tissues (Boyd *et al.* 1994, 2002; Pollard and Baker 1997). In selenium hyperaccumulator plants such as *Stanleya pinnata* (Figure 4), selenium toxicity also reduces herbivory by generalist invertebrate and vertebrate herbivores (Freeman and Pilon-Smits unpublished). On the other hand, certain herbivores appear to have evolved nickel or selenium tolerance. These species are found predominantly on nickel- or selenium-containing plants and accumulate levels of nickel or selenium that are considered toxic to most generalists (Boyd 2002; Freeman and Pilon-Smits unpublished). Such specialist herbivores may affect ecosystem-level processes by mobilizing toxic elements into food webs, as was shown for selenium (Bañuelos *et al.* 2002) and nickel (Peterson *et al.*

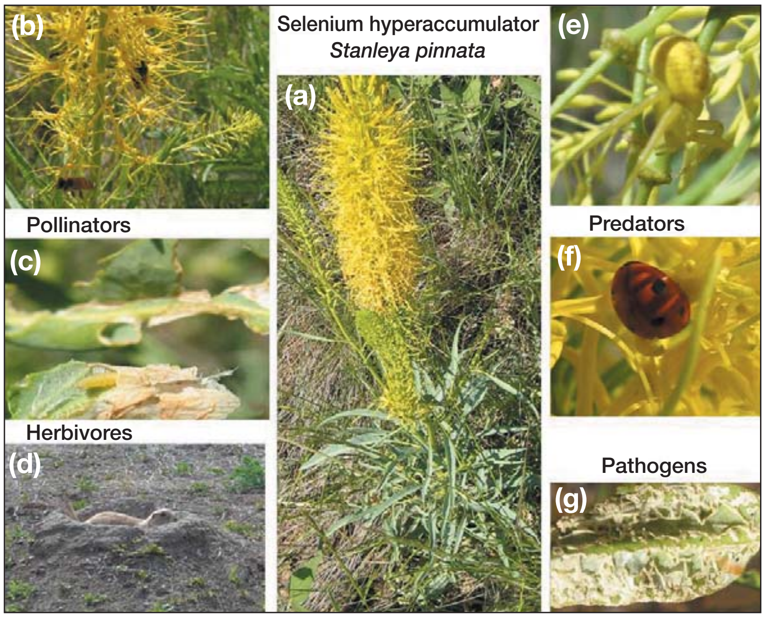

Figure 4. *(a) The selenium hyperaccumulator* Stanleya pinnata *(prince's plume) growing in its native habitat (here, Fort Collins, CO) and some of its ecological partners: (b) honey bee; (c) diamondback moth larva; (d) prairie dog; (e) golden rod spider; (f) ladybug; and (g) white rust.*

2003). Metal accumulation in specialist herbivores may affect their ecological interactions at higher trophic levels via predator deterrence and toxicity, as suggested for selenium in a study by Vickerman *et al.* (2003), and as was found for a nickel-accumulating specialist herbivore (Boyd 2002).

These results from hyperaccumulator plant studies may give some insight into the potential ecological effects of the use of metal accumulating phytoremediator plants. Not much is known about the effects of phytoremediation on the movement of pollutants in the food chain when compared to alternative remediation methods or to no remediation. However, some studies have shown that herbivores were less likely to feed on agricultural plants with elevated levels of selenium, even when concentrations were well below those of hyperaccumulator plants (Trumble *et al.* 1998; Vickerman and Trumble 1999; Vickerman *et al.* 2002; Hanson *et al.* 2003, 2004). It is therefore feasible that long-term phytoextraction projects using plants with high tissue levels of inorganic pollutants will alter the local invertebrate species composition, and perhaps even lead to evolution of metal tolerance in certain fast-evolving invertebrates.

■ How may transgenic plants influence ecological relationships?

In addition to the primary question addressed above (ie what influence phytoremediation may have on ecological relationships), we can ask a secondary question: how might transgenic plants influence these ecological relationships?

Potentially, the escape of transgenic plants or genes could result in a competitive advantage under local conditions. These risks may be alleviated by careful choice of plant material and management practices that minimize risks (Wolfenbarger and Phifer 2000). Escape of the transgene via pollen can be avoided by choosing species that do not have wild relatives. Also, genes may be incorporated into transgenics that prevent them from producing viable pollen or seed. Genes may also be inserted into the chloroplast genome rather than the nuclear genome, so that they can be dispersed only via the maternal line and not in pollen (Ruiz *et al.* 2003). Gene flow via pollen or seeds can also be controlled through management practices such as clipping before flowering, and by planting a non-transgenic buffer zone around the transgenics, as is currently done with such crops. The plants may also be contained in enclosed spaces such as large greenhouses or underground structures. An example is shown in Figure 5, where transgenics are being tested for the cleanup of metal-contaminated mine drainage water in an existing mine drainage tunnel. Large enclosed spaces have also been used for rhizofiltration of radionuclide-contaminated water (Dushenkov and Kapulnik 2000). When assessing the risk of transgene escape, one should not only take into account the probability of escape, but also the potential consequences: will the transgenics have any selective advantage, and if so, in which environments (Wolfenbarger and Phifer 2000)? Insect-resistant transgenics, such as those currently being used in agriculture, will have a clear selective advantage in any environment, and will probably affect herbivore reproduction. Plants engineered to be more tolerant to pollutants may have an advantage in polluted areas, but not in pristine conditions.

Although the use of transgenics for phytoremediation brings with it the added potential risk of escape, it may reduce other risks. If the transgenics are more efficient at detoxifying the pollutant they will reduce exposure of other organisms to that pollutant. Also, more efficient phytoextraction will reduce the time needed for cleanup, and thus pollutant exposure time. Of course, if transgenics have enhanced accumulation of pollutants, they may pose more of a threat to ecological partners if they are consumed. During this time, entry into the food chain may be minimized by fencing, netting, and wildlife repellents (eg scarecrows, periodic noise). Pollutant entry into the food chain may also be prevented in part by the herbivore deterrence effect of the pollutant itself, as was observed for a variety of inorganic elements such as cadmium, zinc, nickel, and selenium (Boyd and Martens 1994; Pollard and Baker 1997; Boyd *et al.* 1999; Hanson *et al.* 2003, 2004).

■ Conclusions

A polluted site poses a risk to the environment. This risk is correlated with the toxicity and concentration of the pollutant, the likeliness of its mobilization and spread by water and wind, and the proximity of sensitive ecosystems. The remediation strategies available for site cleanup will vary in their effectiveness in alleviating the existing risks and in the characteristics of their associated risks, and will also have different timelines and price tags. For each individual site, these initial risks will need to be evaluated in order to design an optimal remediation approach. Once the remediation strategy is decided, steps must be taken to lessen the associated risks. In the case of phytoremediation, careful choice of plant species and management practices are key to promoting ecological restoration and preventing pollutant dispersal. Where possible, native plant species with effective remediation properties and that provide natural hydraulic control (eg trees) and soil stabilization (eg grasses) should be selected. Drip irrigation can be used to prevent leaching, and fencing will minimize pollutant entry into the food chain.

Phytoremediation is an interdisciplinary technology that will benefit from research in many different areas. Much still remains to be discovered about the biological processes that underlie a plant's ability to detoxify and accumulate pollutants. Better knowledge of the biochemical mechanisms involved may lead to: (1) the identification of novel genes and the subsequent development of transgenic plants with superior remediation capacities; (2) a better understanding of the ecological interactions involved (eg plant–microbe interactions); (3) the effect of the remediation process on the existing ecological iinteractions; and (4) the entry and movement of the pollutant in the ecosystem. In addition to being desirable from a fundamental biological perspective, this knowledge will help improve risk assessment during the design of remediation plans (including the additional risks of transgenic plants) as well as alleviation of the associated risks during remediation.

Figure 5. *Transgenic* Brassica juncea *(Indian mustard) grown in a contained hydroponic setup (a mine drainage tunnel in Leadville, CO) to test the altered plants' capacity to remove metals from polluted water.*

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