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# Ecological Risks of Novel Environmental Crop Technologies Using Phytoremediation as an Example

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# ABSTRACT

Phytoremediation is the use of living plants, known as hyperaccumulators which absorb unusually large amounts of metals in comparison to other plants. The use of classical plant breeding and new molecular techniques offers great potential to develop crops with the ability to clean up polluted sites. While these technologies have gained widespread attention, prior to commercial development, there are risks that must be considered – only a few of which have received even modest examination. Therefore, the focus of this working paper is to explore specific risks associated with phytoremediation and suggest ways in which these risks can be managed so that new, novel, and innovative plant technologies may be applied to provide low cost and efficient environmental solutions.

Keywords: risk, GMO, biotechnology, phytoremediation, phytoextraction, phytomining

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# Ecological Risks of Novel Environmental Crop Technologies Using Phytoremediation as an Example

J. Scott Angle[1] and Nicholas A. Linacre[2]

## 1. INTRODUCTION

Phytoremediation is the use of living plants, known as hyperaccumulators which absorb unusually large amounts of metals in comparison to other plants[3], for *in situ* remediation of contaminated soil, sludges, sediments, and groundwater through contaminant removal, degradation, or containment (Baker et al. 1994; Chaney,1983a; Glass 1999). While these technologies have gained widespread attention, prior to commercial development, there are risks that must be considered – only a few of which have received even modest examination. Therefore, the focus of this working paper is to explore specific risks associated with phytoremediation.

Phytoremediation offers the possibility of addressing an intractable global problem by providing an alternative, cheap and effective technology that could significantly improve the prospects of cleaning-up metal contaminated sites (Garbisu and Alkorta 2001; Salt et al. 1995). The advantages of phytoremediation over traditional methods of remediation are well known. The many benefits of the technology have been reviewed by Wolfe and Bjornstad (2002). However, few attempts to assess the specific risks of the technology have been reported.

Theoretical aspects of the risk analysis process were recently reviewed by Linacre et al. (2003). The primary conclusion of this paper was that risks must be identified,

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[3] http://www.epa.gov/tio/download/remed/phytoresgude.pdf

quantified, managed and communicated if phytoremediation is going to find broad public acceptance. Indeed, as a corollary, some of the current concerns related to use of genetically modified agricultural commodities arise from past lack of appreciation for risk assessment, management and communication (MacKenzie 1994). Acceptance of genetically modified organisms (GMOs), at least in the US, has been directly attributed to the eventual public understanding of risks and benefits.

Risk may be defined as the likelihood of occurrence of a negative consequence (Kaplan and Garrick 1981). In the context of phytoremediation, risks are primarily the result of exposure of living organisms to metals. Risk, therefore, depends on the likelihood of exposure, the level of exposure and on the toxic effects of exposure. It is the combination of these factors that dictates the risk which can vary from negligible to high when the likelihood, level and consequences of exposure are significant. Using a source-pathway-receptor model to identify possible risks, the failure to clean up contaminated sites (source) could lead to risk or harm to plants, animals, humans and natural resources such as water (receptors) via significant pollutant linkages (pathways).

Risks in phytoremediation can arise from a number of potential sources. Some risks relate to the direct exposure to metals, while other risks relate to the preparation, cultivation and disposal of materials. This review is divided into a number of sections and discusses: (1) preplanting risks created by soil preparation, (2) risks associated with the transfer of planting materials, (3) potential ecotoxicity, (4) potential weediness, (5) gene flow and introgression, (6) and (7) cultivation risks, (8) volunteers, (9) “additional” risks associated with GMO phtyoremediators, and (10) biomass disposal.

## 2. PRE-PLANT CONSIDERATIONS

Prior to planting of hyperaccumulators, soils are often prepared by adding materials to reduce soil pH. Numerous studies have shown that most hyperaccumulators take up more metal at low pH due to enhanced solubility (Marschner 1995). pH reduction is therefore considered a critical component to most phytoremediation technologies. The only exception to this rule was reported by Li et al. (2003) and Kukier et al. (2004) who showed that maximum uptake of Ni by *Alyssum murale* was at pH greater than 7.5.

However, low pH in the presence of high metal content can exasperate ecotoxicity. Numerous studies have shown that metals can be toxic to most non-hyperaccumulator plants (and even some hyperaccumulators) when soil pH is reduced to 6.0 and below (Chaney 1983b). Further, many soil bacteria and other organisms are sensitive to high metal concentrations which are also affected by lowering pH of high metal soils.

This risk is currently a focus of an on going program in our laboratory. We examined the extent of damage done to an ecosystem when soil pH was reduced for a high metal soil, and whether this damage was permanent or if the ecosystem could be restored to normal by subsequent increase in the soil pH at the end of phytoremediation. Results demonstrated that both microbial number and function are highly sensitive to the toxic effects of soil metals at low soil pH. When soil pH was returned to more appropriate agronomic levels, most parameters, but not all, returned to normal within a six month period.

Reducing pH of low metal soil can also enhance metal solubility to the point where leaching is a concern. Angle et al. (unpublished data) has shown that when soil pH is reduced to values less than 6.4 for Cd and 4.7 for Zn enriched soils, loss from soil via leaching is a significant concern. Metal contaminated groundwater is one of the most difficult media to remediate and should be avoided at all possible costs.

Another soil management option that has been examined is to add organic chelators to soil to increase solubility and thus metal uptake (Blaylock et al. 1997; Lombi et al. 2001). Both aminopolycarboxylic acids (Shen et al. 2002; Huang et al. 1997) and organic acids (Huang et al. 1998; Ebbs et al. 1998) have been studied for their ability to increase metal uptake into plants. While occasionally effective (Meers et al. 2004), these materials have significant limitations that until now have restricted their utility. For both materials, enhanced leaching of chelate-bound metals into groundwater as a result of increased solubility is an important concern (Cunningham et al. 1997; Kedziorek and Bourg 2002). Greman et al. (2001) showed that EDTA added to soil increased leaching losses of metals by up to 40%. One of the paramount rules of remediation is to avoid contaminant dispersal. While it might be possible to balance chelate addition to soil so that only metal taken up by the plant is available at any point in time, the temporal considerations related to full season phytoremediation suggest this to be quite difficult.

A further potential concern is the cost of adding amendments to soil. Sulfur added to lower soil pH is relatively cost effective and thus insignificant to the overall cost of phytoremediation. However, chelates can be quite expensive. Chaney et al. (2004) has reported that the cost of adding 10 mmol EDTA $kg^{-1}$ soil is about $30,000 per ha.

## 3. NATURALLY OCCURRING HYPERACCUMULATORS

Hyperaccumulators are either direct seeded into contaminated or mineralized soils or plantlets are transplanted into the soil. In either case, seed or soils associated with transplants can carry soil bacteria, fungi and viruses that are not indigenous to the area being phytoextracted, potentially posing risks to soil microflora and indigenous plants. However, risk management approaches exist such as soil sterilization and growing plantlets in locally collected soils. One consequence of this approach that is difficult to mitigate the impacts of deliberately introduced soil bacteria important for phytoremediation.

Abou-Shanab et al. (2003) showed that the rhizosphere of *Alyssum murale*, the most important Ni hyperaccumulator studied to date, has bacteria within its rhizosphere that can increase Ni solubility and thus Ni uptake into the plant. While the presence of Ni solubilizing bacteria in soil is beneficial in that phytoremediation efficiency is increased, if these bacteria were also to increase uptake into non hyperaccumulator plants, results could be harmful to the food chain. Abou-Shanab et al. (2003) reported that specific bacteria when inoculated into soil increase Ni uptake by up to 33%. Other studies have shown that the rhizosphere of hyperaccumulators increases metal uptake into plants, although specific reasons for this observation were not considered (Schwartz et al. 2003; Whiting et al. 2001).

## 3. ECOTOXICITY

During phytoremediation, hyperaccumulators are direct seeded or transplanted into metal enriched soil. Early in the process, plant biomass content is low, and ecotoxicity is seldom a concern. However, soon after germination or planting, metal content in biomass increases to levels that can potentially be toxic to the ecosystem. Several studies have shown that hyperaccumulators fail to grow well when foliar concentrations of select metals are low (Li et al. 2004). Li et al reported that *Thlaspi caerulescens* needs 1000x more Zn to grow 'normally' when compared to non-hyperaccumulators. For example, *T. caerulescens* can concentrate up to 40,000 ug $g^{-1}$ Zn (Brown et al. 1994, 1995) while *A. murale* can accumulate similar amounts in above ground biomass (Chaney et al. 2000). Although the exact reason for metal hyperaccumulation has yet to be fully understood, many scientists believe this phenotype evolved as a way of induced foliar toxicity and thus reduce feeding by a variety of insects (Boyd and Martens 1994).

Several studies have examined impacts of high metal biomass on insects. This information has been extensively reviewed by Boyd and Martens (1992 1998). Boyd et al. (1998) examined direct toxic effects on high metal biomass on both feeding preference and toxicity on insects. It was shown that most insect species prefer low metal biomass when given a choice in feeding studies. Less direct effects on reproductive success are more poorly understood. Effects related to behavior may be important but have not yet been examined to our knowledge.

Interestingly, some insects seem to have developed methods for avoiding metal exposure. Aphids for example, due to rapid pass through of fluids, are able to excrete much of the metal consumed during feeding from the phloem. Further, phloem sap generally has lower metal content compared to materials flowing up into the shoot given that this is an important part of the hyperaccumulation process.

We are not aware of any studies that have directly examined toxicity to higher animals consuming high metal hyperaccumulator biomass. However, it is quite reasonable to assume that that exposure to and consumption of high metal biomass could be toxic to wildlife. During large-scale phytoremediation, it will be nearly impossible to fence out all herbivores. We are also aware of concerns related to farm animals getting into fields of hyperaccumulators. Fencing, screens and netting are generally ineffective in preventing movement of all animals into a field in phytoremediation.

Fortunately, most hyperaccumulators are relatively unpalatable probably due to the high alkaloid and metal content found in hyperaccumulators. Thus, while it might be possible that animals will graze on hyperaccumlators, the likelihood of significant consumption is minimal. Our observations of large fields planted to hyperaccumulators are that few, if any, large herbivores forage in these fields. Only when few other foods are present can animals be expected to ingest significant quantities of hyperaccumulator biomass.

Despite the relative unpalatibility of high metal hyperaccumulators, it is always possible that insects could develop resistance to metals, and thus increase feeding and exposure. This has been observed recently for Bt crops as well as for a host of more traditional insecticides.

Further, while most large herbivores have a large grazing range and can simply move to other locations, small herbivores and territorial animals may not have this opportunity. Voles, shrews etc have very limited foraging range and may simply not have the ability to move beyond the confines of a field planted contiguously to hyperaccumulators. Given quantities of roots, stems and leaves consumed and the average metal content of hyperaccumulators, it is readily conceivable that these animals could ingest potentially lethal amounts of metal.

There are no current standards for maximum ingestion of metals by wildlife, but such data are available for livestock. Table 1 (Madejon, et al. 2002) shows the maximum levels of metals tolerated by common livestock species. Hyperaccumulator concentrations for all metals far exceed levels considered toxic to cattle, sheep, swine and chickens, often by orders of magnitude.

**Table 1--Metal concentrations for 'typical' agronomic plants, phytotoxic metal concentrations in plants, metal concentrations used for delineation of hyperaccumulators and maximum metal concentrations tolerated by livestock.**

| Metal | Plant avg. | Phytotoxicity | Hyperacc | Max. conc. tolerated by animals | | |
|---|---|---|---|---|---|---|
| | -----------------mg kg$^{-1}$-------------------- | | | ----------mg kg$^{-1}$ in diet---------- | | |
| | | | | Cattle | Sheep | Chicken |
| Cd | 0.1 – 1 | 5 – 700 | >1,000 | 0.5 | 0.5 | 0.5 |
| Cu | 3 – 20 | 25 – 40 | >10,000 | 100 | 25 | 300 |
| Mn | 15 – 150 | 400 – 2,000 | >10,000 | 1,000 | 400 | 2,000 |
| Ni | 0.1 – 5 | 50 – 100 | >10,000 | 50 | 100 | 300 |
| Zn | 15 – 150 | 500 – 1,500 | >10,000 | 300 | 1,000 | 1,000 |

Adapted from Madejon et al. 2002

Acknowledging that some herbivores with limited foraging range may be killed or injured by the consumption of hyperaccumulators, this hazard must be assessed in terms of risks of doing nothing or the risks of more traditional methods of dig and haul. Doing nothing is often not an option while dig and haul, with replacement of the contaminated soil – and resident small herbivores, will almost certainly be fatal to all wildlife of limited foraging range. Loss of some small herbivores might well be a ‘cost’ of return of the soil ecosystem to a healthy state.

If hyperaccumulator consumption by wildlife is a concern, there are well-established methods to reduce wildlife ingestion of hyperaccumulators. Fencing, deterrents such as periodic noise, and planting of offensive plant species can all be used to reduce contact between wildlife and hyperaccumulators. While not fully effective, these methods will at least reduce exposure.

## 4. ‘WEEDINESS’

Most hyperaccumulators evolved under extreme conditions thus they tend to be quite hardy. Many of these plants evolved on soils that were highly infertile with little water holding capacity. The climate under which many hyperaccumulators evolved is often extreme with cool moist winters and hot and dry summers (Brooks 1998). Our experience has been that hyperaccumulators are quite hardy and survive with little care. Indeed, this is one of the characteristics that make these plants amendable to cultivation.

By definition, most hyperaccumulators are ‘weeds’, in that they: 1) reproduce rapidly, 2) grow under conditions of low fertility, and 3) are adapted to a wide range of environmental (soil and climate) conditions. Consequently one of the most immediate concerns related to phytoremediation is the potential escape of hyperaccumulators from the site of remediation and the possibility that these plants will become environmental weeds.

Numerous examples documenting the ‘escape’ of plants moved from one location to another have been reported. Kudzu, autumn olive, multiflora rose, and Japanese honeysuckle were all intentionally imported into the US, often for agricultural or ornamental purposes. Currently the US spends over $15B annually to control weeds. Most weeds were originally imported into the US either intentionally or accidentally. Most hyperaccumulators will not be used commercially where they evolved, thus import into other areas will be necessary. However, importing a non-indigenous species into many countries can be a problem. Therefore the first choice when selecting a plant species for use is to select an indigenous hyperaccumulator.

A more general notion of a weed is that of "a plant growing in a place where it is not wanted." In this sense evidence exists that the physiological limitations of hyperaccumulator plants may limit their potential for weediness. Many hyperaccumulators have been reported to survive only on metal enriched soils. It has been suggested that high shoot metal content, and subsequent toxicity to pathogens and insects is one of the primary reasons why metals are accumulated (Pollard et al. 2002). When hyperaccumulators were grown in low metal soil, we have observed that plants rapidly die from fungal disease. Most often, we have identified *Pythium* or *Phythopera* root disease as the causative agent of death and decline.

It is suggested that hyperaccumulators have abandoned other methods of protection from disease for genetic efficiency. Thus, when metals are low, uptake is reduced and the plants are left 'defenseless'. For this reason, it is unlikely that hyperaccumulators that escape from the site of cultivation would survive to become a permanent component of the ecosystem. Plants would most likely die as a result of being left without the high metal defense. We, therefore, do not believe that control using herbicides will be necessary to protect against escape outside areas of metal contamination or artificial enrichment.

Even if movement of seed is restricted beyond the original area of establishment, pollen from hyperaccumulators can travel with wind and insects for many kilometers. Numerous crop species have been shown to hybridize with wild relatives, including sunflower, radish, canola and millet. Traits that increase gene flow and outcrossing include self-incompatibility, high outcrossing rates and biotic pollination. *Thlaspi* is generally considered to be self-pollinating but cross-pollination ranges from 5 to 25%. This concern will be discussed in more detail in the next section.

## 5. GENE FLOW AND INTROGRESSION

Pollen dispersal may be critical because it affects the likely breadth of dispersal of genetic material containing metal accumulating genes. It has been reported that the primary method by which genes may move from a GMO crop to a weedy relative is through pollen movement (Kareiva et al. 1994). It might be possible that genes coding for metal uptake and sequestration could be transferred to other crop or non crop plants. For crop plants, transfer of high metal uptake is a very serious concern. Incorporation of metal uptake genes into crops plants could lead to food and feed that exceed national and international standards for metal concentration. In addition to food quality concerns, export markets can be negatively affected by high metal content.

The primary method for flow of genes from both GMO and non-GMO crops to related relatives is through the process of introgression. Introgression is defined as the natural spread of genes of one species into another through the process of interspecific hybridization followed by successive backcrosses to the parent. This process results in offspring that have similar genotypes to the wild type but incorporate new genes from the domesticated, exotic or GM plants.

Most plant species outcross to some extent with other plants. Although *T. caerulescens* was originally thought to have only limited outcrossing, i.e. approximately 5% (Riley 1956) more recent evidence has suggested that outcrossing for this species might be much higher. Koch (1998) demonstrated that outcrossing rates in *T. caerulescens* could reach as high as 88%. It has also been suggested that higher outcrossing may occur on contaminated soils compared to plants grown on less polluted soil (Dubois et al. 2003).

Just about every cultivated crop has shown hybridization with at least one wild relative (Arias and Rieseberg 1994; National Research Council 1989). Sexually compatible weeds or indigenous species almost always occur within the growing area of most known cultivated plant species. For this reason, outcrossing during phytoremediation should be anticipated and controlled to the extent possible.

There are many factors that can affect the flow of pollen and genes from one plant to another – some of which can potentially be managed and reduced. The most important factors affecting gene flow include the degree of out-crossing and the potential for biotic pollen movement. In general, high out-crossing rates and biotic pollination, such as with bees, will increase the rate of gene flow between plant species. Pollen can be dispersed by a variety of vectors: wind, insects, mammals and birds. The role different vectors play in long versus short distance dispersal is species specific. And, in the case of GM crops, the type of gene may affect the rate of introgression. Glover (2002) cites the example of insect protected cotton where there has been a 37-54 percent reduction in insecticide use. This in turn may increase local insect populations, increasing the abundance of pollen vectors therefore increasing the rate of introgression.

## 6. CULTIVATION ON CONTAMINATED AREAS

For many soil metal contaminants, especially Zn and Ni, within a metal contaminated area, hyperaccumulator plants are likely to do well since foliar concentrations will be high. This may result in the plants spreading within the contaminated area and displacing other “indigenous” species. However, diversity on contaminated sites is typically low with the more aggressive species being dominant and many species previously growing on contaminated sites are themselves considered weeds. Therefore since this is an area needing remediation, spreading within the contaminated area and displacement of other “indigenous” species is generally considered a positive attribute.

On the other hand, for metals such as Cd in contaminated or mineralized soils, soil concentrations never or rarely approach levels that are toxic to plants. Cadmium tends to have few effects on plant growth, yet can still exhibit toxicity to animals. While this does not obviate the ecotoxicity of high Cd plants, Cd hyperaccumulators will not be able to grow beyond the area of soil contamination. Thus, escape of Cd hyperaccumulators beyond the area of contamination is not likely to be a concern. These observations suggest that escape from the original site must be assessed based upon both the plant and soil.

## 7. CULTIVATION ON NATURALLY ENRICHED AREAS

Cultivation of hyperaccumulators on naturally enriched areas offers the greatest promise for use in phytomining. Phytomining is a more specific form of phytoremediation where the purpose of metal removal from soil is economic gain. For example, millions of acres of Ni rich ultramafic soil are found around the world. These soils are potentially amendable to Ni phytomining. However, many of these areas are populated by a number of rare and endangered species. For example, serpentine soils in northern California and southern Oregon are populated by rare and endemic species that exist only on these soils (Kruckeberg 1954;1984). Given the unique flora of enriched soils, concern has been raised that highly competitive and aggressive introduced hyperaccumulators may displace some of the natural flora. The literature is replete with examples of introduced plant species dominating fragile ecosystems. We have experience working on a serpentine pine barren. Pines were never part of the indigenous ecosystem, and only came to dominate as part of human activity (clear cutting). This has threatened much of the indigenous flora in serpentine areas and has led to debate as to whether pines should be removed by logging.

As discussed, concerns related to escape of introduced hyperaccumulators exist and must be addressed prior to project initiation. It is therefore critical to establishment a protocol to monitor the potential for escape from the original area of introduction. Where escape in found, survival should be observed to determine whether this is temporary or whether escaped hyperaccumulators have the potential to become established as a permanent component of the ecosystem.

Angle et al. (2001) has reported that many hyperaccumulators are readily controlled with herbicides. Therefore, where escape is found, it is possible to control these individuals with herbicides. However, this is both an expensive and time consuming process and there can be no guarantee that all escaped plants will be found and killed.

Another method to reduce the potential for escape from the original site of planting is to harvest plants prior to seed set. Most hyperaccumulators set seed in mid summer. Since most hyperaccumulators are perennials, they will typically be harvested at the time of maximum metal accumulation, then plants will continue to grow for an additional harvest the same year or in the following year. Fortunately, maximum metal accumulation usually occurs just about at the time of flowering. It is therefore possible to harvest plants before seed are produced. Alternatively, for locations where it is unlikely that plants will survive the entire year (i.e. growing temperate hyperaccumulators in tropical areas), plants can be grown as annuals and again harvested before seed set.

## 8. VOLUNTEERS

After remediation is complete there remains the limited potential for some seed, stored in the soil seed bank, to germinate, which may pose a small risk. However, this risk may be managed using on site volunteer management. Different approaches are available and will depend on the crop. Generally, rotation with another crop, against which any phytoremediation volunteers will be visually obvious, allows the volunteers to be identified and removed. The potential for volunteers can also be reduced by harvesting before seed set, which reduces the likelihood of seed entering the soil seed bank.

## 9. GMO HYPERACCUMULATORS

Genetic studies related to hyperaccumulators have been underway for many years Karenlämpi et al. 2000; Whiting et al. 2004). Most studies have focused on the identification of genes involved in the process of hyperaccumulation – uptake, transport and sequestration (Rutherford, et al. 2004; Wang et al. 2002). However, a few studies have resulted in the development of GMO hyperaccumulators, hopefully with enhanced potential to extract metals from soil. Dhankher et al. (2002) reported the development of a plant with enhanced tolerance and uptake of arsenic. More recently, several studies have described transgenic plants with the ability to take up and volatize selenium (Van Huysen et al. 2003; 2004). *Brassica juncea* was engineered to over express a key enzyme in the sulfur assimilation pathway, which resulted in significantly greater uptake of Se. Probably the greatest amount of study, and resulting publicity has occurred for poplar trees engineered for Hg uptake. Bizily et al. (2001, 2003) and Pilon-Smits and Pilon (2000) studied the insertion of genes that could potentially enhance Hg uptake and volatilization. This work has successfully resulted in the production of transgenic trees that increase Hg uptake and volatilization from soil. Similar reports that transgenic plants can increase Se uptake and volatilization have also recently been reported (Pilon-Smits et al. 1999a, 1999b).

While the genomics of phytoremediation is proceeding at a rapid pace, concerns have been raised regarding this approach. Imagine the public reaction to the following GMO hyperaccumulator:

> “A non indigenous, weed that has been genetically engineered to be poisonous to most organisms that come into contact with it.”

Each of the attributes noted above (weediness, non-indigenous import, poisonous, and GMO) raises serious individual concerns. Multiplying each of these risks together might be more than the public is willing to tolerate. Multiplication of concerns only exasperates the need to study, discuss and balance potential concerns with potential benefits.

Two general approaches are current underway to develop GMO hyperaccumulators. The first approach, which is by far the less common of the two, is to move genes that code for ‘large’ plant growth into true hyperaccumulators. Most, but certainly not all, hyperaccumulators are small plants with low biomass. Since phytoremediation efficacy is a function of both biomass produced and biomass metal content, most consider the small size of these plants to be the primary limiting factor. By moving these genes into small hyperaccumulators, the goal is to create a GMO hyperaccumulator that expresses higher biomass production. This approach might find greater use in the future since genes that code for enhanced growth are relatively well characterized. This is in contrast to genes that code for metal hyperaccumulation which are not yet fully understood and at best appear to be controlled by at least several genes.

The most significant concern with this approach is the subsequent transfer of genes that code for large plant growth from the GMO hyperaccumulator to local weeds. As previously noted, it is likely that plants within the same genus as the GMO hyperaccumulator will be found in the area under phytoremediation. Most of these plants can be weeds under the right conditions. For this reason, all practices to control introgression should be used.

Another approach for control that could potentially be used is to create sterile plants via the insertion of suicide genes. Suicide genes (DeBlock and Debrouwer 1993; Strauss et al. 1995) have been used for a variety of crops, primarily to prevent seed production that could be saved, thus leading to loss of control of the intellectual property (this could also be used to prevent hyperaccumulator GMOs from inappropriate use that violates the original patent).

The second approach for the development of GMO hyperaccumulators and the one that is under active investigation is to identify genes that enhance metal uptake and transfer these genes to plants with much higher biomass. Most studies previously discussed are following this approach. The most important question for this approach is the selection of the crop plant that will be engineered for enhanced metal uptake. It is very important that that we avoid introduction of metal-accumulation genes into crop plants that could either escape or via introgression spread them into nearby crop plants. We have spent decades trying to keep excess metals out of crop plants in order to protect human and animal health as well as to protect export markets (Chaney et al. 2001). Now we are inserting genes that enhance metal uptake of crop plants to potentially toxic levels.

One final concern that has been raised in several public forum is that enhance uptake and volatilization of volatile metals (Hg and Se) might exasperate downwind air pollution. While volatilization is certainly a consequence of Hg and Se removed from soil (and is also a natural soil process), the overall contribution to air in comparison to amounts released via volatilization by indigenous bacteria is minor. And since many areas beyond several interior California valleys are Se-deficient, increased aerial deposition might be a positive attribute of the process since downwind soil concentrations might actually be increased.

## 10. BIOMASS DISPOSAL

Once plants are harvested, biomass must be either disposed of using appropriate techniques or recycled to recover valuable metals. Most phytoremediation scenarios envision that the biomass will be incinerated either to reduce volume, recover energy or both. However, burning of metals, regardless of the form it is in, can lead to the formation of metal oxides. Some metals are extremely volatile (Hg and Cd) while others belong to an intermediate group (Zn and Pb). Metals such as Ni, Cr. and Cu are considered non volatile (Belevi and Moench 2000). Metal oxides are both toxic and carcinogenic. Thus, care must be taken to control emissions during the incineration or smelting process. Numerous methods are available to reduce gas emissions yet all are very expensive.

Ash that results from the burning process can contain as high as 30% metal on a weight basis. This concentration is several times higher than hard rock ore mined from the ground. Thus, the 'bio-ash' or 'bio-ore' is a rich and potentially valuable ore depending upon the price of the extracted metal. Through smelting or electro-wining processes, metals can be extracted from the bio-ore (Prasad and Freitas 2003; Kumar et al. 1995).

A concern that has yet to be adequately addressed is the potential toxic nature of either biomass or bio-ore. Biomass can contain up to 4% and bio-ore can contain up to 30% metal on a dry weight basis. Does either of these values cause the material to be classified as a hazardous waste? The questions remains unresolved in the US, yet for countries like Switzerland, the high metal concentration of the burned biomass would clearly classify the ash as a hazardous waste, and thus restrict ash disposal in a landfill (Swiss Federal Legislation 1996). For this reason, high metal biomass would have to be incinerated with low metal materials (such as municipal solid waste) to dilute metals to acceptable levels.

The USEPA classifies a hazardous waste as: “by-products of society that can pose a substantial or potential hazard to human health or the environment when improperly managed. The waste must also pose at least one of four characteristics (ignitability, corrosivity, reactivity or toxicity), or appears on special EPA lists.”

The confusion relates to the source of the metal in the hyperaccumulator. For example, high Ni in soil can result either from mining and smelting operations or occur naturally in soil from mineralization of ultramafic minerals. Nickel removed from naturally enriched soil should not be considered a pollutant and thus guidelines that govern hazardous waste need to be questioned. This conflict has yet to be addressed in a regulatory forum. Nickel extracted from soil contaminated by anthropogenic activities might better fall within a regulatory agency thus needing more through review.

Hazardous wastes are subject to a variety of rules and regulations, especially as related to transport. It is theoretically possible that the bio-ore resulting from incineration could be classified as a hazardous waste. If true, simple transport of the bio-ore will be subject to a variety of Department of Transportation regulations. At best, the burning of biomass and generation of the bio-ore will be conducted all on a single site. This might even be the smelter that originally caused the contamination. At worst, the biomass and or bio-ore might need to be transported via public roads to other locations.

## 11. CONCLUSIONS

In conclusion, there are real risks associated with phytoremediation that require assessment and identification of management options prior to implementation of any field based operations. Management options using confinement strategies such as onsite processing, discing, harvesting before seed set, and volunteer management, may reduce the likelihood of pollen and seed movement thus reducing potential risks. Data collection, interpretation and communication of risks must be evaluated if phytoremediation is going to find wide public acceptance. This argues for a balanced approach in the discussion of the benefits and risks of phytoremediation.

In any discussion of risks, however, specific risks must be considered in comparison to doing nothing – leaving the site unaltered. Risks of phytoremediation must also be assessed compared to the more traditional methods of remediation including, excavation and landfilling, soil incineration, soil washing and vitrification (EPA 1997, MADEP 1993). Traditional methods of remediation have many real risks, both to human and environmental health that must also be considered. Thus, while acknowledging that there are risks associated with phytoremediation, these risks are temporary that last only during the process of phytoremediation. We believe that in most cases phytoremediation risks are small compared to the risks of doing nothing or the financial and engineering risks of ‘dig and haul.’

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