PHYTOREMEDIATION LITERATURE REVIEW

by

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Pollution and Environmental Awareness in The United States

The 20th century can be characterized as a time of increasing environmental awareness. Much of the society came to realize that in the race for progress and prosperity, it failed to protect the environment and the natural resources on which it depends. In the earlier half of the 20th century, the disposal of industrial waste by many companies in the United States was regarded as a non-productive function to be achieved at the least possible cost (Cook, 1977). This mentality, coupled with insufficient governmental action and legislation, led to massive contamination of groundwater and soil at sites across the United States (Ward, 1999). In the latter half of the 20th Century people witnessed such environmental disasters as the pollution of Lake Erie and Lake Ontario (International Joint Commission, 1970), the discovery of toxic waste under the Love Canal community of New York (Levine, 1982), and the smog-related deaths of more than 4,000 people in London (Wise, 1968). Such widespread pollution gained considerable public attention and brought about monumental changes in American society.

Positive steps were taken in the United States during the late 1960's and early 1970's to raise public awareness and to curtail environmental pollution by implementing stringent governmental regulations. The Solid Waste Disposal Act of 1965 (SWDA) was the first act that regulated waste on a national scale (Reed et al., 1992). In 1969, Congress passed the National Environmental Policy Act (NEPA), the first act to provide a national policy for the environment. The first annual Earth Day was held on April 22, 1970, to celebrate the environment and to heighten public awareness of the problems that compromise the integrity of the environment. In the same year, President Richard Nixon established the Environmental Protection Agency (EPA) as the implementing arm of the NEPA. Other important legislation of the 1970's included the Clean Air Act (CAA; 1970), the Federal Water Pollution Control Act (FWPCA; 1972), the Safe Drinking Water Act (SDWA; 1974), and the Resource Conservation and Recovery Act (RCRA; 1976). As stated by Reed et al. (1992), these acts and others passed by Congress provided for the “cradle to the grave” regulation of hazardous waste. Congress later passed the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, commonly called Superfund; 1980) that enabled the federal government to delegate the costs of remedial action to the parties responsible for hazardous waste violations. Pressure to meet the new standards for environmental quality propelled whole industries to re-engineer their fundamental processes and products (Cunningham et al., 1997) and forced some companies out of business (Cammarota, 1980). The proper disposal of hazardous waste and the need to clean existing contaminated sites became a productive function for many public and private institutions in light of the substantial fines and penalties, which could be mandated by regulatory agencies. Government agencies and private industry alike began a search for efficient, cost-effective technologies that could be used to remediate (i.e. clean) hazardous waste sites, an initiative that remains to the present day.

Currently 300,000 to 400,000 hazardous waste sites in the United States require some future remedial action (NRC, 1997). However, only an estimated 30,000 of these are recognized by the EPA as candidates for immediate treatment (Ensley, 2000). These sites may be polluted with inorganic contaminants, organic contaminants, or more commonly mixtures of both. The remediation of all U.S. hazardous waste sites in existence could cost as much as $1 trillion (NRC, 1997), but the estimated expense for sites of immediate concern is much less. The projected cost for remediation of areas containing mixtures of heavy metals and organic pollutants is $35.4 billion over the next five years, whereas cleanup of sites contaminated with metals only would cost $7.1 billion (Ensley, 2000). The high cost of hazardous waste cleanup is due in part to the inefficiency and high cost of available technologies. Conventional remediation techniques are based on civil and chemical engineering technologies including a wide variety of physical, thermal, and chemical treatments, as well as manipulations to accelerate or reduce mass transport in the contaminated matrix (Cunningham et al., 1997). According to NRC (1997), as cleanup at waste sites has proceeded, it has become evident that despite the billions of dollars invested, conventional remediation technologies are inadequate. The lack of commercially available technologies that can restore contaminated sites at reasonable cost has led to increasing pressure to limit waste cleanups to sites that pose immediate risks to human health.

Metals and other inorganic contaminants are among the most prevalent forms of contamination found at waste sites, and their remediation in soils and sediments are among the most technically difficult (Cunningham et al., 1997). Sources of anthropogenic metal contamination include smelting of metalliferous ore, electroplating, gas exhaust, energy and fuel production, the application of fertilizers and municipal sludges to land, and industrial manufacturing (Blaylock and Huang, 2000; Cunningham et al., 1997; Raskin et al., 1994). Heavy metal contamination of the biosphere has increased sharply since 1900 (Nriagu, 1979) and poses major environmental and human health problems worldwide (Ensley, 2000). According to Raskin et al. (1994), the term heavy metal is arbitrary and imprecise. In this dissertation, 'heavy metal' will refer to any element that has metallic properties and atomic number greater than 20 (Raskin et al., 1994). Unlike many organic contaminants, most metals and radionuclides cannot be eliminated from the environment by chemical or biological transformation (Cunningham and Ow, 1996; NRC, 1997). Although it may be possible to reduce the toxicity of certain metals by influencing their speciation, they do not degrade and are persistent in the environment (NRC, 1999). The following section describes some of the various conventional remediation technologies that are used to clean heavy metal polluted environments. Each method has specific benefits, limitations, and costs (Table 1), which should be considered by those responsible for remedial action.

Conventional Remediation Technologies

Excavation and Landfill

Excavation of soil followed by disposal in a landfill is the most commonly used method of cleaning sites that have been contaminated with heavy metals (Begonia et al., 1998). A major criticism of this method is that contaminants are merely moved from one site to another with no effort to destroy, remove, or stabilize them on site. Containment measures at the landfill are designed to isolate the contaminated material from the environment so that any liquid or gaseous interchange is minimized or controlled (Wood, 1997). Other remediation techniques are commonly used at landfill sites to aid in the isolation of hazardous materials. For instance, landfill caps reduce the amount of water infiltration and suppress the downward migration of contaminants, whereas underground vertical barriers inhibit lateral movement.

Impermeable or Containment-type Barriers

Impermeable barriers can be used to completely surround a source of groundwater contamination and are among the least expensive, most widely used means of preventing the spread of metals in groundwater (NRC, 1999). As previously mentioned, barriers are used often at landfills to isolate the contaminated mass from the outside environment (Wood, 1997). This method may include the use of caps, horizontal or vertical walls, or a combination of these. According to Wood (1997), a cap consists of a single or multiple layers of un-contaminated material that covers an area of contamination. The primary function of a cap is to suppress the downward migration of metals by controlling the infiltration of water. Covering the area also prevents the exposure of at-risk targets and encourages vegetative growth over the site. The cap can be vegetative or consist of certain clays, lime, fly ash, sewage sludge, concrete or asphalt, or synthetic membranes or geotextiles. Underground vertical barriers are used around the perimeter of a contaminated site to control the lateral movement of groundwater. Vertical barriers can be composed of clay mixtures, concrete, steel sheet piling, and synthetic membranes (Wood, 1997). Horizontal walls function in a similar manner as caps in preventing the downward migration of contaminants. However, horizontal barrier technology is not yet perfected and is seldom used due to the inherent difficulties in establishing an impenetrable layer under a site. Because contaminants are not removed, sites where barrier technologies have been used will often have long-term site restrictions, something that parties responsible for site restoration must consider.

Permeable Reactive Barriers

A permeable reactive barrier is a passive treatment zone of reactive material which is installed across the flow path of a contaminant plume (NRC, 1999). As groundwater flows through the treatment zone, metal contaminants are immobilized by sorption or precipitation within the barrier. Reactive barriers can be constructed of any materials that react with inorganic contaminants including zeolites, hydrous ferric oxide, peat, silica, polymer gels, or limes. They are most often used to treat localized areas where contaminant plumes exist but can be used to totally enclose an area where the movement of contaminants off site poses high risk to the surrounding environment. Sorption or precipitation within a reactive barrier must be regarded as a retardation of contaminant migration rather than as a permanent solution to the problem (NRC, 1999).

In Situ Vitrification

In situ vitrification is a remediation technology used to treat small areas with high levels of organic or inorganic soil contamination. Soils are heated to temperatures between 1000 and 1700°C and are melted by applying an alternating electrical current between electrodes placed in the ground. When cool, soil becomes an impermeable glass or crystalline solid which is more resistant to leaching of the chemically or physically bound metals than the original soil (NRC, 1999; Wood, 1997). The vitrified material may be covered with clean soil, and left on site or removed and disposed of in a controlled landfill. This expensive remedial technology is normally reserved for contamination that is not readily treated by other methods (Wood, 1997).

Solidification and Stabilization

Solidification and stabilization technologies are is designed to suppress the movement of contaminants in soils, sludges, and liquids by reducing their solubility or by changing the permeability of the matrix (NRC, 1997). The objective is to stabilize contaminants by binding them physically within a solidified mass, which is more resistant to leaching than the original soil. The success of this technology depends on the ability to mix the stabilizing agent with the contaminated matrix (NRC, 1999). The principal stabilization materials are portland-type cements, pozzolanic materials, lime, silicates, clays, and polymers (Wood, 1997; NRC, 1999). Pozzolans are small spherical fly ash particles formed in the combustion of coal. Those that are high in silica have cement-like properties when mixed with water (Wood, 1997). If left on site, the solidified monolith may require long-term monitoring to ensure that leaching of contaminants does not occur.

In Situ Redox Manipulation

In situ redox manipulation is used to reduce the mobility or toxicity of certain metals that are hazardous in their oxidized form but not in reduced form (NRC, 1999). This manipulation can be used to treat metals in soil and groundwater that are not readily accessible from the surface. This method involves the injection of chemical reducing agents into the ground or the stimulation of naturally-occurring iron-reducing bacteria with nutrients, to create reducing conditions in the subsurface. Commonly used reducing agents include aluminum, sodium and zinc metals, and some specific iron compounds (Wood, 1997). Long-term monitoring and treatment may be required to ensure that mobilization of the contaminants does not occur by reoxidation (NRC, 1999).

Soil Washing

A problem with the excavation-and-landfill method is that the majority of the soil mass being deposited in a landfill consists of soil components themselves and not the actual pollutant. Not only is it expensive to place large volumes of soil in a controlled landfill, it also reduces the amount of space available for other hazardous materials. Soil washing is a soil remediation technique that aims to concentrate soil contaminants into a relatively small volume (Wood, 1997). The benefit of consolidating hazardous substances is that costs associated with disposal and treatment are related only to the reduced volume of process residues. After excavation, contaminated soil is taken to a washing facility where it is screened to remove debris and large objects and then leached with washing agents such as acids or chelates which displace or extract contaminants from soil particles (Dennis et al., 1994; NRC, 1999). The resulting leachate is rich in the target contaminant and can be treated as waste water (Wood, 1997), a less expensive approach than the disposal of the soil itself.

Soil Flushing

The goal of soil flushing is identical to that of soil washing, to liberate contaminants from the solid phase of a soil and concentrate them in a liquid phase which can be recovered and treated as waste water. Both techniques employ the use of washing or extracting solutions, but soil flushing is an *in situ* process whereas soil washing takes place off site. The process of soil flushing involves the use of extracting chemicals, which are applied to the contaminated soil by surface flooding, sprinklers, leach fields, or by vertical or horizontal injection walls (NRC, 1999). After contact with the soil, the flushing solutions are recovered for disposal or treatment. Soil flushing is a clean-up method, which is seldom used by itself. Other remediation techniques that specialize in the recovery of the contaminant-rich leachate will commonly be used in conjunction with soil flushing.

Electrokinetic Systems

Some contaminated soils are not suitable for soil flushing because of their low permeability or because of the perceived difficulties in recovering the extracting solution. Electrokinetic systems employ the use of electrical fields to mobilize and remove contaminants in soil and are attractive alternatives to soil flushing for low permeability soils (EPA, 1997). As described by Wood (1997), this is an *in situ* process where an electrical current is passed through an array of electrodes that are embedded in the soil. When the current is applied, contaminants move through soil water in pore spaces towards the electrode of opposite charge. The electrodes have porous housings into which purging solutions are pumped to remove the contaminants and bring them to the surface. The purging solutions are then taken to a water treatment plant for contaminant removal. Site managers must be prepared to handle the large amounts of acid and base which are produced by the process. The efficiency of this technique has not been proven in the United States and will require further field testing before it becomes an accepted remediation method by regulatory agencies.

Bioremediation

The term bioremediation is sometimes thought to be synonymous with phytoremediation, but these terms describe two completely different methods. Although both seek to exploit living organisms to alter contaminated environments, bioremediation involves the manipulation of microbial populations, and phytoremediation concerns the use of higher plants. Bioremediation refers to a process through which metal contaminants are modified as a direct result of microbial activity (NRC, 1999). The objective may be to mobilize, immobilize, or reduce the toxicity of metals in soil or water depending on the ultimate goals of remediation (Smith et al., 1994). If reducing conditions are maintained by the addition of suitable substrates, such as oxygen and nutrients, inorganic contaminants will remain in their highly insoluble, immobile forms (NRC, 1999; Wood, 1997). However, the immobilization of some contaminants should be viewed as a temporary fix and not a final solution to the problem.

Unconventional Plant-based Remediation Technologies: The Phytoremediation Concept

Development of Phytoremediation

Conventional remediation technologies are used to clean the vast majority of metal-polluted sites. The reason is because they are fast, relatively insensitive to heterogeneity in the contaminated matrix, and can function over a wide range of oxygen, pH, pressure, temperature, and osmotic potentials (Cunningham et al., 1997). However, they also tend to be clumsy, costly, and disruptive to the surrounding environment (Cunningham and Ow, 1996). Of the disadvantages of conventional remediation methods, cost (Table 1) is the primary driving force behind the search for alternative remediation technologies. Some micro-organism-based remediation techniques, such as bioremediation, show potential for their ability to degrade and detoxify certain contaminants. Although these biological systems are less amenable to environmental extremes than other traditional methods, they have the perceived advantage of being more cost-effective (Cunningham et al., 1997). Bioremediation is most applicable for sites that have been contaminated with organic pollutants, and as such, this condition has been the focus of the majority of bioremediation research. Because heavy metals are not subject to degradation, several researchers have suggested that bioremediation has limited potential to remediate metal-polluted environments. In contrast, plants are known to sequester certain metal elements in their tissues (Marschner, 1995) and may prove useful in the removal of metals from contaminated soils (Chaney, 1983). Over the past decade there has been increasing interest for the development of plant-based remediation technologies which have the potential to be low-cost, low-impact, visually benign, and environmentally sound (Cunningham and Ow, 1996), a concept called phytoremediation.

Phytoremediation is a word formed from the Greek prefix "phyto" meaning plant, and the Latin suffix "remedium" meaning to clean or restore (Cunningham et al., 1997). The term actually refers to a diverse collection of plant-based technologies that use either naturally occurring or genetically engineered plants for cleaning contaminated environments (Flathman and Lanza, 1998). The primary motivation behind the development of phytoremediative technologies is the potential for low-cost remediation (Table 1; Ensley, 2000). Although the term, phytoremediation, is a relatively recent invention, the practice is not (Brooks, 1998a; Cunningham et al., 1997). Research using semi-aquatic plants for treating radionuclide-contaminated waters existed in Russia at the dawn of the nuclear era (Salt et al., 1995a; Timofeev-Resovsky et al., 1962). Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity (Baker and Brooks, 1989; Baker et al., 1991; Reeves and Brooks, 1983). Chaney (1983) was the first to suggest using these "hyperaccumulators" for the phytoremediation of metal-polluted sites. However, hyperaccumulators were later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal (Comis, 1996; Cunningham et al., 1995; Ebbs et al., 1997). By definition, a hyperaccumulator must accumulate at least 1000 $\mu g \cdot g^{-1}$ of Co, Cu, Cr, Pb, or Ni, or 10,000 $\mu g \cdot g^{-1}$ (i.e. 1%) of Mn or Zn in the dry matter (Reeves and Baker, 2000; Wantanabe, 1997). Some plants tolerate and accumulate high concentrations of metal in their tissue but not at the level required to be called hyperaccumulators. These plants are often called moderate metal-accumulators, or just moderate accumulators (Kumar et al., 1995). The lack of viable plant alternatives for phytoremediation seemed to suppress the amount of phytoremediation research conducted between the mid 1980s and the early half of the 1990s. The search for plants for phytoremediation centered on the Brassica family to which many hyperaccumulators belong (Cunningham et al., 1995). Through the work of various researchers, particularly Kumar et al. (1995) and Dushenkov et al. (1995), several high-biomass, metal-accumulating species were identified. Phytoremediation research gained momentum after the discovery of these plants, and most of our understanding of this emerging technology has come from research reports published since 1995.

Phytoremediation consists of a collection of four different plant-based technologies, each having a different mechanism of action for the remediation of metal-polluted soil, sediment, or water. These include:

rhizofiltration, which involves the use of plants to clean various aquatic environments; phytostabilization, where plants are used to stabilize rather than clean contaminated soil; phytovolatilization, which involves the use of plants to extract certain metals from soil and then release them into the atmosphere through volatilization; and phytoextraction, where plants absorb metals from soil and translocate them to the harvestable shoots where they accumulate. Although plants show some ability to reduce the hazards of organic pollutants (Carman et al., 1998; Cunningham et al., 1995; Gordon et al., 1997), the greatest progress in phytoremediation has been made with metals (Blaylock and Huang, 2000; Salt et al., 1995a; Watanabe, 1997). Phytoremediative technologies which are soil-focused are suitable for large areas that have been contaminated with low to moderate levels of contaminants. Sites which are heavily contaminated cannot be cleaned through phytoremediative means because the harsh conditions will not support plant growth. The depth of soil which can be cleaned or stabilized is restricted to the root zone of the plants being used. Depending on the plant, this depth can range from a few inches to several meters (Schnoor et al., 1995). Phytoremediation should be viewed as a long-term remediation solution because many cropping cycles may be needed over several years to reduce metals to acceptable regulatory levels. This new remediation technology is competitive with, and may be superior to existing conventional technologies at sites where phytoremediation is applicable. Phytoremediation is not the solution for all hazardous waste problems but is rather a tool that can be used, possibly in conjunction with other clean-up methods, to remediate polluted environments.

Rhizofiltration

Metal pollutants in industrial-process water and in groundwater are most commonly removed by precipitation or flocculation, followed by sedimentation and disposal of the resulting sludge (Ensley, 2000). A promising alternative to this conventional clean-up method is rhizofiltration, a phytoremediative technique designed for the removal of metals in aquatic environments. The process involves raising plants hydroponically and transplanting them into metal-polluted waters where plants absorb and concentrate the metals in their roots and shoots (Dushenkov et al., 1995; Flathman and Lanza, 1998; Salt et al., 1995a; Zhu et al., 1999). Root exudates and changes in rhizosphere pH also may cause metals to precipitate onto root surfaces. As they become saturated with the metal contaminants, roots or whole plants are harvested for disposal (Flathman and Lanza, 1998; Zhu et al., 1999). Most researchers believe that plants for phytoremediation should accumulate metals only in the roots (Dushenkov et al., 1995; Flathman and Lanza, 1998; Salt et al., 1995a). Dushenkov et al. (1995) explains that the translocation of metals to shoots would decrease the efficiency of rhizofiltration by increasing the amount of contaminated plant residue needing disposal. In contrast, Zhu et al. (1999) suggest that the efficiency of the process can be increased by using plants which have a heightened ability to absorb and translocate metals within the plant. Despite this difference in opinion, it is apparent that proper plant selection is the key to ensuring the success of rhizofiltration as a water cleanup strategy.

Dushenkov and Kapulnik (2000) describe the characteristics of the ideal plant for rhizofiltration. Plants should be able to accumulate and tolerate significant amounts of the target metals in conjunction with easy handling, low maintenance cost, and a minimum of secondary waste requiring disposal. It is also desirable for plants to produce significant amounts of root biomass or root surface area. Several aquatic species have the ability to remove heavy metals from water, including water hyacinth (*Eichhornia crassipes* (Mart.) Solms; Kay et al., 1984; Zhu et al., 1999), pennywort (*Hydrocotyle umbellata* L.; Dierberg et al., 1987), and duckweed (*Lemna minor* L.; Mo et al., 1989). However, these plants have limited potential for rhizofiltration, because they are not efficient at metal removal, a result of their small, slow-growing roots (Dushenkov et al., 1995). These authors also point out that the high water content of aquatic plants complicates their drying, composting, or incineration. Despite limitations, Zhu et al. (1999) indicated that water hyacinth is effective in removing trace elements in waste streams. Terrestrial plants are thought to be more suitable for rhizofiltration because they produce longer, more substantial, often fibrous root systems with large surface areas for metal sorption. Sunflower (*Helianthus annuus* L.) and Indian mustard (*Brassica juncea* Czern.) are the most promising terrestrial candidates for metal removal in water. The roots of Indian mustard are effective in the removal of Cd, Cr, Cu, Ni, Pb, and Zn (Dushenkov et al., 1995), and sunflower removes Pb (Dushenkov et al., 1995), U (Dushenkov et al., 1997a), ^{137}Cs, and ^{90}Sr (Dushenkov et al., 1997b) from hydroponic solutions.

Rhizofiltration is a cost-competitive technology in the treatment of surface water or groundwater containing low, but significant concentrations of heavy metals such as Cr, Pb, and Zn (Table 1; Ensley, 2000). The commercialization of this technology is driven by economics as well as by such technical advantages as applicability to many problem metals, ability to treat high volumes, lesser need for toxic chemicals, reduced volume of secondary waste, possibility of recycling, and the likelihood of regulatory and public acceptance (Dushenkov et al., 1995). However, the application of this plant-based technology may be more challenging and susceptible to failure than other methods of similar cost (Table 1). The production of hydroponically grown transplants and the maintenance of successful hydroponic systems in the field will require the expertise of qualified personnel, and the facilities and specialized equipment required can increase overhead costs. Perhaps the greatest benefit of this remediation method is related to positive public perception. The use of plants at a site where contamination exists conveys the idea of cleanliness and progress to the public in an area that would have normally been perceived as polluted.

Phytostabilization

Sometimes there is no immediate effort to clean metal-polluted sites, either because the responsible companies no longer exist or because the sites are not of high priority on a remediation agenda (Berti and Cunningham, 2000). The traditional means by which metal toxicity is reduced at these sites is by in-place inactivation, a remediation technique that employs the use of soil amendments to immobilize or fix metals in soil. Although metal migration is minimized, soils are often subject to erosion and still pose an exposure risk to humans and other animals. Phytostabilization, also known as phytorestoration, is a plant-based remediation technique that stabilizes wastes and prevents exposure pathways via wind and water erosion; provides hydraulic control, which suppresses the vertical migration of contaminants into groundwater; and physically and chemically immobilizes contaminants by root sorption and by chemical fixation with various soil amendments (Berti and Cunningham, 2000; Cunningham et al., 1995; Flathman and Lanza, 1998; Salt et al., 1995a; Schnoor, 2000). This technique is actually a modified version of the in-place inactivation method in which the function of plants is secondary to the role of soil amendments. Unlike other phytoremediative techniques, the goal of phytostabilization is not to remove metal contaminants from a site, but rather to stabilize them and reduce the risk to human health and the environment.

The most comprehensive and up-to-date explanation of the phytostabilization process is offered by Berti and Cunningham (2000). Before planting, the contaminated soil is plowed to prepare a seed bed and to incorporate lime, fertilizer, or other amendments for inactivating metal contaminants. Soil amendments should fix metals rapidly following incorporation, and the chemical alterations should be long lasting if not permanent. The most promising soil amendments are phosphate fertilizers, organic matter or bio-solids, iron or manganese oxyhydroxides, natural or artificial clay minerals, or mixtures of these amendments.

Plants chosen for phytostabilization should be poor translocators of metal contaminants to aboveground plant tissues that could be consumed by humans or animals. The lack of appreciable metals in shoot tissue also eliminates the necessity of treating harvested shoot residue as hazardous waste (Flathman and Lanza, 1998). Selected plants should be easy to establish and care for, grow quickly, have dense canopies and root systems, and be tolerant of metal contaminants and other site conditions which may limit plant growth. The research of Smith and Bradshaw (1979) led to the development of two cultivars of *Agrostis tenuis* Sibth. and one of *Festuca rubra* L. which are now commercially available for the phytostabilization of Pb-, Zn-, and Cu-contaminated soils.

Phytostabilization is most effective at sites having fine-textured soils with high organic-matter content but is suitable for treating a wide range of sites where large areas of surface contamination exist (Berti and Cunningham, 2000; Cunningham et al., 1995). However, some highly contaminated sites are not suitable for phytostabilization, because plant growth and survival is not a possibility (Berti and Cunningham, 2000). At sites which support plant growth, site managers must be concerned with the migration of contaminated plant residue off site (Schnoor, 2000) or disease and insect problems which limit the longevity of the plants. Phytostabilization has advantages over other soil-remediation practices in that it is less expensive, less environmentally evasive, easy to implement, and offers aesthetic value (Berti and Cunningham, 2000; Schnoor, 2000). When decontamination strategies are impractical because of the size of the contaminated area or the lack of remediation funds, phytostabilization is advantageous (Berti and Cunningham, 2000). It may also serve as an interim strategy to reduce risk at sites where complications delay the selection of the most appropriate technique for the site.

Phytovolatilization

Some metal contaminants such as As, Hg, and Se may exist as gaseous species in environment. In recent years, researchers have searched for naturally occurring or genetically modified plants that are capable of absorbing elemental forms of these metals from the soil, biologically converting them to gaseous species within the plant, and releasing them into the atmosphere. This process is called phytovolatilization, the most controversial of all phytoremediation technologies. Mercury and Se are toxic (Suszcynsky and Shann, 1995; Wilber, 1980), and there is doubt about whether the volatilization of these elements into the atmosphere is safe (Watanabe, 1997). Selenium phytovolatilization has been given the most attention to date (Banuelos et al., 1993; Lewis et al., 1966;

McGrath, 1998; Terry et al., 1992), because this element is a serious problem in many parts of the world where there are areas of Se-rich soil (Brooks, 1998b). However, there has been a considerable effort in recent years to insert bacterial Hg ion reductase genes into plants for the purpose of Hg phytovolatilization (Bizily et al., 1999; Heaton et al., 1998; Rugh et al., 1996, 1998). Although there have been no efforts to genetically engineer plants which volatilize As, it is likely that researchers will pursue this possibility in the future.

According to Brooks (1998b), the release of volatile Se compounds from higher plants was first reported by Lewis et al. (1966). Terry et al. (1992) report that members of the Brassicaceae are capable of releasing up to 40g Se $ha^{-1}day^{-1}$ as various gaseous compounds. Some aquatic plants, such as cattail (*Typha latifolia* L.), are also good for Se phytoremediation (Pilon-Smits et al., 1999). Unlike plants that are being used for Se volatilization, those which volatilize Hg are genetically modified organisms. *Arabidopsis thaliana* L. and tobacco (*Nicotiana tabacum* L.) have been genetically modified with bacterial organomecurial lyase (*merB*) and mercuric reductase (*merA*) genes (Heaton et al., 1998; Rugh et al., 1998). These plants absorb elemental Hg(II) and methyl mercury (MeHg) from the soil and release volatile Hg(O) from the leaves into the atmosphere (Heaton et al., 1998).

The phytovolatilization of Se and Hg into the atmosphere has several advantages. Volatile Se compounds, such as dimethylselenide, are 1/600 to 1/500 as toxic as inorganic forms of Se found in the soil (DeSouza et al., 2000). The volatilization of Se and Hg is also a permanent site solution, because the inorganic forms of these elements are removed and the gaseous species are not likely to be redeposited at or near the site (Atkinson et al., 1990; Heaton et al., 1998). Furthermore, sites that utilize this technology may not require much management after the original planting. This remediation method has the added benefits of minimal site disturbance, less erosion, and no need to dispose of contaminated plant material (Heaton et al., 1998; Rugh et al., 2000). Heaton et al. (1998) suggest that the addition of Hg(O) into the atmosphere would not contribute significantly to the atmospheric pool. However, those who support this technique also agree that phytovolatilization would not be wise for sites near population centers or at places with unique meteorological conditions that promote the rapid deposition of volatile compounds (Heaton et al.,1998; Rugh et al., 2000). Unlike other remediation techniques, once contaminants have been removed via volatilization, there is a loss of control over their migration to other areas. Despite the controversy surrounding phytovolatilization, this technique is a promising tool for the remediation of Se and Hg contaminated soils.

Phytoextraction

Phytoextraction is the most commonly recognized of all phytoremediation technologies, and is the focus of the research proposed in this prospectus. The terms phytoremediation and phytoextraction are sometimes incorrectly used as synonyms, but phytoremediation is a concept while phytoextraction is a specific cleanup technology. The phytoextraction process involves the use of plants to facilitate the removal of metal contaminants from a soil matrix (Kumar et al., 1995). In practice, metal-accumulating plants are seeded or transplanted into metal-polluted soil and are cultivated using established agricultural practices. The roots of established plants absorb metal elements from the soil and translocate them to the above-ground shoots where they accumulate. If metal availability in the soil is not adequate for sufficient plant uptake, chelates or acidifying agents may be used to liberate them into the soil solution (Huang et al., 1997a; Lasat et al., 1998). After sufficient plant growth and metal accumulation, the above-ground portions of the plant are harvested and removed, resulting the permanent removal of metals from the site. As with soil excavation, the disposal of contaminated material is a concern. Some researchers suggest that the incineration of harvested plant tissue dramatically reduces the volume of the material requiring disposal (Kumar et al., 1995). In some cases valuable metals can be extracted from the metal-rich ash and serve as a source of revenue, thereby offsetting the expense of remediation (Comis, 1996; Cunningham and Ow, 1996). Phytoextraction should be viewed as a long-term remediation effort, requiring many cropping cycles to reduce metal concentrations (Kumar et al., 1995) to acceptable levels (Table 2). The time required for remediation is dependent on the type and extent of metal contamination, the length of the growing season, and the efficiency of metal removal by plants, but normally ranges from 1 to 20 years (Blaylock and Huang, 2000; Kumar et al., 1995). This technology is suitable for the remediation of large areas of land that are contaminated at shallow depths with low to moderate levels of metal- contaminants (Kumar et al., 1995; Wantanabe, 1997).

Many factors determine the effectiveness of phytoextraction in remediating metal-polluted sites (Blaylock and Huang, 2000). The selection of a site that is conducive to this remediation technology is of primary importance. Phytoextraction is applicable only to sites that contain low to moderate levels of metal pollution, because plant growth is not sustained in heavily polluted soils. Soil metals should also be bioavailable, or subject to absorption by plant roots. The land should be relatively free of obstacles, such as fallen trees or boulders, and have an acceptable topography to allow for normal cultivation practices, which employ the use of agricultural equipment. As a plant-based technology, the success of phytoextraction is inherently dependent upon several plant characteristics. The two most important characters include the ability to accumulate large quantities of biomass rapidly and the ability to accumulate large quantities of environmentally important metals in the shoot tissue (Blaylock et al., 1997; Cunningham and Ow, 1996; Kumar et al., 1995; McGrath, 1998). It is the combination of high metal accumulation and high biomass production that results in the most metal removal. Ebbs et al. (1997) reported that *B. juncea*, while having one-third the concentration of Zn in its tissue, is more effective at Zn removal from soil than *T. caerulescens*, a known hyperaccumulator of Zn. This advantage is due primarily to the fact that *B. juncea* produces ten-times more biomass than *T. caerulescens*. Plants being considered for phytoextraction must be tolerant of the targeted metal, or metals, and be efficient at translocating them from roots to the harvestable above-ground portions of the plant (Blaylock and Huang, 2000). Other desirable plant characteristics include the ability to tolerate difficult soil conditions (e.g., soil pH, salinity, soil structure, water content), the production of a dense root system, ease of care and establishment, and few disease and insect problems. Although some plants show promise for phytoextraction, there is no plant which possesses all of these desirable traits. Finding the perfect plant continues to be the focus of many plant-breeding and genetic-engineering research efforts.

Enhancing the Phytoextraction Process

Increasing Metal Availability in Soil

A major factor influencing the efficiency of phytoextraction is the ability of plants to absorb large quantities of metal in a short period of time. Hyperaccumulators accumulate appreciable quantities of metal in their tissue regardless of the concentration of metal in the soil (Baker, 1981), as long as the metal in question is present. This property is unlike moderate accumulators now being used for phytoextraction where the quantity of absorbed metal is a reflection of the concentration in the soil. Although the total soil metal content may be high, it is the fraction that is readily available in the soil solution that determines the efficiency of metal absorption by plant roots. To enhance the speed and quantity of metal removal by plants, some researchers advocate the use of various chemicals for increasing the quantity of available metal for plant uptake. Chemicals that are suggested for this purpose include various acidifying agents (Blaylock and Huang, 2000; Brown et al.,1994; Cunningham and Ow, 1996; Huang et al., 1998), fertilizer salts (Lasat et al., 1997;1998) and chelating materials (Blaylock et al., 1997; Huang et al., 1997a). These chemicals increase the amount of bioavailable metal in the soil solution by either liberating or displacing metal from the solid phase of the soil or by making precipitated metal species more soluble. Research in this area has been moderately successful, but the wisdom of liberating large quantities of toxic metal into soil water is questionable.

Soil pH is a major factor influencing the availability of elements in the soil for plant uptake (Marschner, 1995). Under acidic conditions, H^+ ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from sesquioxides and variable-charged clays to which they have been chemisorbed (i.e. specific adsorption; McBride, 1994). The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption. Many metal cations are more soluble and available in the soil solution at low pH (below 5.5) including Cd, Cu, Hg, Ni, Pb, and Zn (Blaylock and Huang, 2000; McBride, 1994). It is suggested that the phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil (Brown et al., 1994; Blaylock and Huang, 2000; Salt et al., 1995a). Possible amendments for acidification include NH_4-containing fertilizers, organic and inorganic acids, and elemental S. Trelease and Trelease (1935) indicated that plant roots acidify hydroponic solutions in response to NH_4 nutrition and cause solutions to become more alkaline in response to NO_3 nutrition. Metal availability in the soil can be manipulated by the proper ratio of NO_3 to NH_4 used for plant fertilization by the effect of these N sources on soil pH, but no phytoremediation research has been conducted on this topic to date. The acidification of soil with elemental S is a common agronomic practice, which can be used to mobilize metal cations in soil. Brown et al. (1994) acidified a Cd- and Zn-contaminated soil with elemental S and observed that accumulation of these metals by plants was greater than when the amendment was not used. Acidifying agents are also used to increase the availability of radioactive elements in the soil for plant uptake. Huang et al. (1998) reported that the addition of citric acid increases U accumulation in Indian mustard (*B. juncea*) tissues more than nitric or sulfuric acid although all acids decrease soil pH by the same amount. These authors speculated that citric acid chelates the soil U, thereby enhancing its solubility and availability in the soil solution. The addition of citric acid causes a 1000-fold increase of U in the shoots of *B. juncea* compared to accumulation in the control (no citric acid addition). Despite the promise of some acidifying agents for use in phytoextraction, little research is reported on this subject.

The addition of chelating materials to soil, such as EDTA, HEDTA, and EDDHA, is the most effective and controversial means of liberating labile metal-contaminants into the soil solution. Chelates complex the free metal ion in solution, allowing further dissolution of the sorbed or precipitated phases until an equilibrium is reached between the complexed metal, free metal, and insoluble metal fraction (Norvell, 1991). Chelates are used to enhance the phytoextraction of a number of metal contaminants including Cd, Cu, Ni, Pb, and Zn (Blaylock et al., 1997; Huang et al., 1997a, 1997b). Huang et al. (1997a) suggested that chelates are able to induce Pb accumulation in agronomic crops such as corn (*Zea mays* L.) and pea (*Pisum sativum* L.). These authors reported a 1000-fold increase of Pb in the soil solution after HEDTA application in comparison to soil solution of a control (no HEDTA addition). Under these conditions Pb concentrations in the shoots of corn and pea increases from less than 500 $mg \cdot kg^{-1}$ to more than 10,000 $mg \cdot kg^{-1}$ within one week after HEDTA application. This chelate-assisted accumulation of toxic quantities of metal in a non-accumulator species is termed "chelate-induced hyperaccumulation" (Huang et al., 1997a). These researchers explained that when chelate-induced hyperaccumulation is the goal, metals on site are initially immobilized to allow for rapid establishment and growth of an agronomic crop such as corn. When the crop accumulates sufficient biomass, chelating materials are applied to the soil to result in the liberation of large quantities of metal into the soil solution. Massive amounts of metal are absorbed by plant roots and are translocated to the shoot tissue where they accumulate to toxic levels. After death, plants are harvested and removed from the site. Chelate-induced hyperaccumulation is in contrast to the normal practice of phytoextraction where plants are given a gradual exposure to non-toxic quantities of metal in solution, and accumulation occurs gradually over time as the plants grow. The controversy surrounding the use of chelates deals with the fate of the residual chelate in the soil after metal absorption occurs (Brooks, 1998a). The massive liberation of chelate-bound metals into the soil solution makes them subject to leaching into deeper soil layers. Metals which migrate downward beyond the root zone of plants cannot be recovered through means of phytoremediation and may require the use of more expensive conventional remediation methods. The primary concern is that the liberated metals have the ability to migrate into uncontaminated areas, possibly groundwater reservoirs (Cunningham et al., 1997). The scientific literature lacks appreciable information concerning the appropriate amount of chelate to apply under different levels of contamination and for different plant species. Further research is required to determine the fate of the chelate-metal complex in soil before the use of these amendments are accepted widely for use in phytoextraction.

Some positively charged metals and radionuclides may be bound to the soil CEC by weak electrostatic forces and may be displaced by other cations in the soil solution (Sparks, 1995). High-valence cations with a low degree of hydration are preferentially adsorbed to the cation-exchange sites than cations with low valence and a high degree of hydration (hierarchy is shown in the lyotrophic series).

Lyotrophic Series:

$$Al^{3+}>Pb^{2+}>Sr^{2+}>Ca^{2+}>Ni^{2+}>Cd^{2+}>Cu^{2+}>Co^{2+}>Zn^{2+}>Mg^{2+}>Ag^{+}>Cs^{+}>Rb^{+}>K^{+}>NH_4^{+}>Na^{+}$$

Because the binding preference is also concentration dependent, it is possible for a cation lower in the binding hierarchy to displace others that are adsorbed more strongly to the exchange sites (Sparks, 1995). Lasat et al. (1997) indicated that the application of NH_4 (as NH_4NO_3) or K (as KNO_3) increases Cs desorption from soil and increased its accumulation in the tissue of cabbage (*Brassica oleracea* L.), tepary bean (*Phaseolus acutifolius* A. Gray.), and Indian mustard (*B. juncea*). These authors reported that the desorption of Cs is concentration-dependent, increasing with NH_4 and K concentrations up to 0.2 molar. Similarly, Dushenkov et al. (1999) find that NH_4-containing salts are the most practical in liberating soil-bound Cs in terms of efficiency and cost. The application of this phytoextraction method in the field has not been successful in recent experiments. Lasat et al. (1998) report that the addition of NH_4NO_3 to a Cs-contaminated soil does not significantly increase in the level of Cs in the shoots of plants being tested. It is speculated that the free Cs quickly migrated out of the root zone, resulting in decreased accumulation of Cs in plant tissues. It is also possible that the applied NH_4 was rapidly converted into NO_3 through the process of nitrification (Marschner, 1995), resulting in less dissolution of Cs from the soil. The use of fertilizer salts to increase the bioavailability of contaminants for plant extraction may be a promising phytoextraction technique, but further research is required to demonstrate its effectiveness under field conditions. Future research that addresses the use of NH_4-containing fertilizers should incorporate the use of a nitrification inhibitor, such as nitrapyrin (2-chloro-6(trichloromethyl) pyridine), to eliminate nitrification as a source of experimental error.

Proper Plant Selection

As a plant-based technology, the success of phytoextraction is inherently dependent upon proper plant selection. As previously discussed, plants used for phytoextraction must be fast growing and have the ability to accumulate large quantities of environmentally important metal contaminants in their shoot tissue (Blaylock et al., 1997; Cunningham and Ow, 1996; Kumar et al., 1995; McGrath, 1998). Many plant species have been screened to determine their usefulness for phytoextraction. Researchers initially envisioned using hyperaccumulators to clean metal polluted soils (Chaney, 1983). At present, there are nearly 400 known hyperaccumulators (Salt and Kramer, 2000), but most are not appropriate for phytoextraction because of their slow growth and small size. Several researchers have screened fast-growing, high-biomass-accumulating plants, including agronomic crops, for their ability to tolerate and accumulate metals in their shoots (Banuelos et al., 1997; Blaylock et al., 1997; Dushenkov et al., 1995; Ebbs et al., 1997; Ebbs and Kochian, 1997, 1998; Huang et al., 1997a; 1997b; Kumar et al., 1995; Lasat et al., 1997; 1998; Salt et al., 1995b). Many metal-tolerant plant species, particularly grasses, escape toxicity through an exclusion mechanism and are therefore better suited for phytostabilization than phytoextraction (Baker, 1981; Ebbs et al., 1997). However, barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) are tolerant of metals such as Cu, Cd, and Zn, and accumulate moderate to high amounts of these metals in their tissues (Ebbs and Kochian., 1998). Many herbaceous species, including members of the Brassicaceae, also accumulate moderate amounts of various metals in their shoots. A list of promising plant species for phytoextraction of metals and radionuclides is given (Table 3). One of the most promising, and perhaps most studied, non-hyperaccumulator plant for the extraction of heavy metals from contaminated sites is Indian Mustard (*B. juncea*).

Many hyperaccumulators belong to the Brassica family. Once it was suspected that known hyperaccumulators were not suited for phytoextraction, researchers looked to other high biomass-accumulating members of the Brassicaceae for plants which accumulated large quantities of toxic metals (Dushenkov et al., 1995; Kumar et al., 1995). Kumar et al. (1995) tested many fast growing Brassicas for their ability to tolerate and accumulate metals, including Indian mustard (*B. juncea*), black mustard (*Brassica nigra* Koch), turnip (*Brassica campestris* L.), rape (*Brassica napus* L.), and kale (*Brassica oleracea* L). Although all Brassicas accumulated metal, *B. juncea* showed a strong ability to accumulate and translocate Cu, Cr VI, Cd, Ni, Pb, and Zn to the shoots. Kumar et al. (1995) also investigated possible genetic variation of different *B. juncea* accessions in hope of finding some that had more phytoextraction potential than others.

The term, accession, refers to seeds that have been gathered from a particular area and are now part of a collection at a seed bank or plant-introduction station (personal communication; Rick Luhman, curator of Brassicas for the USDA-ARS Plant Introduction Station at Iowa State University). Once in the collection, seeds are assigned a number that identifies the particular accession. Although all Indian mustard accessions are *B. juncea* Czern., they may exhibit different phenotypes as a result of being from different regions where environmental factors may have influenced the natural selection of this species. Kumar et al. (1995) determined that accessions 426308, 211000, 426314, and 182921 are among the best suited for phytoextraction. Several researchers have confirmed the phytoremediation potential of these and other *B. juncea* accessions (Blaylock et al., 1997; Dushenkov et al., 1995; Ebbs and Kochian, 1998; Salt et al., 1995b). The USDA-ARS Plant Introduction Station of Iowa State now maintains, and distributes, metal-accumulating accessions which are considered useful for phytoremediation.

Indian mustard is an oilseed Brassica crop for which cultivation extends from India through western Egypt and Central Asia to Europe (Nishi, 1980). According to Prakash (1980), the oldest reference to *B. juncea* in Sanskrit literature is by the name 'Rajika', and carbonized seeds of this species have been found in the ancient sites of the Harappan civilization (2300-1750 B.C.). Despite the efforts of historians and researchers, the precise origin of this crop remains an enigma. Perhaps the most likely place or places of origin are those regions where its parents, *B. nigra* and *B. campestris*, overlap in their distribution. Possible centers of origin include Africa (Zeven and Zhukovsky, 1975), China (Chen et al., 1995), the Middle East, Southwest Asia, and India (Sauer, 1993). Indian mustard is eaten as a leafy vegetable in China but is grown in India primarily for its oil-containing seeds (~ 40% oil; Prakash, 1980), which serve as a source of cooking oil and spice (Nishi, 1980; Krzymañski, 1997). Indian mustard is capable of producing 18 tons of biomass per hectare per crop (Kumar et al., 1995). Plants perform very well in nutrient solution culture, progressing from the four-leaf stage to fully grown plants (up to 50 g shoot fresh mass) in as little as 21 days (personal observations). Although short-day conditions (<12 hrs light) result in a more compact growth habit (personal observations), shorter height, and limited leaf production (Bhaskar and Vora, 1994), biomass accumulation is greater than under long-day conditions (9-10 hrs light optimal; Neelam et al., 1994). Long-day conditions promote early flowering (Bhaskar and Vora, 1994) but are not required for flower development. These plants have indeterminate growth and continue to branch from the nodes and to accumulate biomass after flower and siliquae (seed pod) development. The recommended fertility rate for maximum growth of *B. juncea* under un-contaminated conditions is 75 to120 kg N ha^{-1} and 30 to 50 kg P_2O_5 per hectare (Gurjar and Chauhan, 1997; Thakral et al., 1995; Tomar et al., 1997). Zaurov et al. (1999) reported that biomass accumulation of *B. juncea* was greatest when plants in soil are supplied with 200 kg N, 100 kg P_2O_5, and 66 kg K_2O per hectare. However, Cd concentration in the tissue was greatest when no N was supplied.

Indian mustard is given considerable attention by present day researchers, geneticists, and plant breeders in particular, because of its unique polyploid genome. *Brassica juncea* is an allotetraploid, a plant with a genome composed of the complete diploid genomes of both parents, *B. nigra* and *B. campestris.* In modern breeding programs, selection of *B. juncea* is based on a wide variety of characters. Improving oil and meal quality by eliminating nutritionally undesirable erucic acid or by modifying the fatty-acid composition of oil is an important objective for some plant breeders (Banga, 1997). Other selections are based on insect (diamondback moth, *Plutella xylostella* (L.); Andrahennadi and Gillott, 1998) and disease resistance (blackleg, *Leptosphaeria maculans* (Desm.) Ces.; Pang and Halloran, 1996) and various temperature adaptations (Banga, 1997). Only recently has there been an interest in selecting Indian mustard lines based on their ability to tolerate and accumulate heavy metals. Several accessions of *B. juncea* have been identified as moderate accumulators of metallic elements and are maintained by the USDA-ARS Plant Introduction Station at Iowa State University, Ames, Iowa. The benefit of using *B. juncea* seed from the plant introduction station is that the genetic integrity of the accessions is preserved through appropriate breeding techniques. Experiments that utilize these seeds have more precision than those conducted with seeds from commercially available sources. Precision is also greater, because future researchers can obtain the same accessions for their experiments. The USDA-ARS Plant Introduction Station maintains a world-wide collection of *B. juncea* accessions that are known metal-accumulators, and the seeds are distributed to public and private research institutions at no cost.

Specialized Agricultural Practices

Part of the enthusiasm for using common agronomic crops for phytoextraction is because their cultivation and growth requirements are well established. However, plant growth will surely differ under contaminated conditions (Blaylock et al., 1997), and established agronomic practices may not elicit the same plant response as under non-contaminated conditions. Much is known about the response of plants to high levels of metallic elements in a root medium (Adriano et al., 1971; Horst and Marschner, 1978; Marschner, 1995; Mengel and Kirkby, 1987; Ulrich, 1976), but most relevant agronomic research aims to decrease plant exposure to these elements. Some agronomists, and all phytoremediation researchers, are interested in promoting plant growth, but those involved with phytoextraction aim to do this while encouraging the accumulation of large quantities of metals within the plant. The goals of traditional agronomy and phytoremediation differ in some areas, and as such, it is necessary to evaluate the suitability of agronomic practices for phytoextraction. By optimizing practices such as irrigation, fertility, planting, and harvest time, it is thought that the efficiency of phytoextraction can be increased (Salt et al., 1995a). The need for specialized agronomic practices is agreed upon by phytoremediation researchers (Brown et al., 1994; Cunningham et al., 1995; Cunningham and Ow, 1996; Ebbs et al., 1997; Huang et al., 1997a; Kumar et al., 1995; Salt et al., 1995a; Schwartz and Morel, 1998), yet few research efforts have addressed this issue directly. This area of phytoremediation offers the greatest opportunity for original research, particularly in the area of plant nutrition and soil fertility.

Fertilizers are used commonly in agriculture to promote plant health and to increase yield, but the benefits and limitations of fertilization with respect to phytoremediation are not clear. Different forms of the same nutrient, such as NH_4 and NO_3, elicit very different responses in plant growth and element absorption by roots and may dramatically affect the chemical nature of the rhizosphere (Barker and Mills, 1980; Trelease and Trelease, 1935). It is important to understand how the concentration and type of nutrients applied influence the phytoextraction process so that effective fertility-management strategies can be established. The identification of nutritional disorders for *B. juncea* and other plants used for phytoextraction will lend insight into which nutrient elements need to be supplied in phytoextraction fertility regimes. It is not known, however, whether or not additions of deficient elements will promote plant growth at the expense of metal accumulation. Plants used for phytoextraction, such as *B. juncea*, may develop nutritional disorders when subjected to elevated levels of metal contaminants, such as Zn, in the root medium (Ebbs and Kochian, 1997), and future research should investigate these and other factors which may limit plant growth. Successful phytoextraction is dependent on the accumulation of plant biomass and on the accumulation of metal within the tissue (Blaylock et al., 1997; Cunningham and Ow, 1996; Kumar et al., 1995; McGrath, 1998). The over application of a deficient element can suppress the absorption of the target element. Proper plant nutrition has the potential to be an effective, low-cost agronomic practice for enhancing the phytoextraction of heavy metals by plants, but more research is required before fertilizers can be used effectively for this purpose.

General Information on Zinc

Zinc is an important element not only because it is essential for animals and plants (Brown et al., 1993; Welch, 1993), but because it has a wide range of industrial uses (Cammarota, 1980). Zinc occurs naturally in many minerals as sulfides, sulfates, oxides, carbonates, phosphates, and silicates (Barak and Helmke, 1993) with the principal ore being sphalerite, a zinc sulfate (Cammarota, 1980). Zinc metal has many uses including galvanizing of metal surfaces, production of zinc-based alloys such as bronze and brass, and the production of zinc chemicals which are used extensively in manufacturing (Cammarota, 1980). Despite its importance in our everyday lives, Zn is the heavy metal occurring in the greatest concentrations in the majority of wastes arising in modern, industrialized communities (Boardman and McGuire, 1990). According to Lambert et al. (1997), the soil is a major sink for Zn, and nearly all contamination of surface soils by Zn is a result of human activity (Chaney, 1993). Anthropogenic sources of contamination arise from activities such as mining and smelting, electroplating and galvanizing, application of industrial and municipal sludges to land, excessive use of Zn-containing agricultural chemicals, and other industrial activities (Blaylock and Huang, 2000; Chaney, 1993; Lambert et al., 1997). The United States was once a major contributor of Zn to the world market, but there has been a marked reduction in smelting activities since 1975, the year that the Environmental Protection Agency (EPA) subjected mining and smelting operations to stringent environmental regulations (Cammarota, 1980).

Zinc can be held to soil component through a variety of mechanisms including cation exchange, specific adsorption (chemisorption), and chelation (Barrow, 1993; McBride, 1994; Shuman, 1980). In general, Zn is more available in the soil solution for plant uptake under acidic conditions than under alkaline conditions (Peech, 1941). According to McBride (1994), the reasons for the increased availability at low pH relate to the influence of the H^+ concentration on the various mechanisms of sorption. Under acidic conditions, the functional groups responsible for specific adsorption may become protonated and create a net positive charge on sesquioxides, amorphous clays, and organic matter, resulting in the release of Zn^{2+} into the soil solution. Hydrogen also has the ability to displace other cations such as Zn^{2+} from the cation exchange complex. Chelation of Zn^{2+} by organic matter is also influenced by soil acidity through its effect on the number of ligands involved in chelation. Monodentate bonds between Zn and the chelating ligands are weak compared with multidentate bonds. Under acidic conditions, the monodentate bonds are more prevalent, and Zn^{2+} can be more easily displaced from these chelate complexes through exchange processes. It is well understood that plants influence soil pH under non-contaminated conditions, particularly in the immediate vicinity of the roots (Marschner, 1995). However, is it not understood how the pH of the root medium is affected by plants that are subjected to Zn contamination under various fertility regimes. This information may help future researchers to anticipate changes in Zn availability in soil, and may lead to more specialized management practices which improve the ability of plants to extract Zn for phytoextraction.

Zinc was determined as an essential element for plant growth in 1926 by Sommer and Lipman (Sommer and Lipman, 1926). This element is primarily taken up as Zn^{2+} but may be taken up as $ZnOH^+$ (Marschner, 1995). In plants, the function of Zn resembles that of Mn and Mg in that it brings about the binding conformation between enzyme and substrate (Mengel and Kirkby, 1987). The metabolic functions of Zn are based on its tendency to form tetrahedral complexes with N-, O-, and particularly S-ligands, and it thereby plays functional (catalytic) and structural roles in enzymatic reactions (Brown et al. 1993; Vallee and Auld, 1990). Zinc plays a functional role in carbonic anhydrase, which catalyzes the conversion of CO_2 to HCO^- (Hatch and Burnell, 1990). The activity of 1, 5-ribulose-bisphosphate carboxylase is also controlled by Zn, an enzyme which catalyzes the initial step of photosynthetic CO_2 fixation in plants (Jyung et al., 1972). Copper and Zn have roles in maintaining the integrity of biomembranes, because they are structural sites components of superoxide dismutase, an enzyme that protects plants from damaging superoxide ($O_2 \cdot^-$) radicals (Brown et al., 1993).

The role of Zn in protein synthesis is linked to its involvement in maintaining the structural integrity of ribosomes, RNA, and DNA. Zinc is also a component of proteins which are involved in the processes of translation and replication (Giedroc et al., 1986; Hanas et al., 1983).

The synthesis of indole acetic acid (IAA; auxin) is also dependent on Zn, but the specific role of this element is not agreed upon by researchers. Tryptophan is the precursor for IAA synthesis, and Zn is considered essential for the synthesis of this amino acid (Tsui, 1948). Others believe that auxin synthesis is influenced by Zn through its role in the biosynthesis of IAA from tryptophan, not because it is essential for tryptophan synthesis (Salami and Kenefick, 1970). The role of Zn in auxin biosynthesis may account for the suppressed root growth observed among plants with Zn toxicity (Mengel and Kirkby, 1987).

According to Marschner (1995), most plants are deficient when Zn levels in the dry mass are between 15 and 30 mg kg^{-1} or less, and toxicity appears in the range of 100 to 300 mg Zn kg^{-1} or higher. Zinc toxicity often leads to leaf chlorosis, which may represent an induced deficiency of another essential element (Marschner, 1995). Because of the similar ionic radius of fourfold coordinated Zn^{2+} (74 pm), Cu^{2+} (71 pm) Fe^{2+} (77 pm) and Mg^{2+} (71 pm), these elements are competitive for absorption by roots, and when available in excess each may induce a deficiency of another (Adriano et al., 1971; Barak and Helmke, 1993; Loneragan and Webb, 1993; Marschner, 1995). Recent evidence also suggests that excessive Zn can reduce the activity of nitrate reductase (Luna et al., 2000) and may therefore impair the ability of plants to use NO_3.

The accumulation and partitioning of Zn in the plant is highly dependent on the supply in the root medium (Longnecker and Robson, 1993). When Zn supply is adequate to toxic, a large portion is bound to the surface of cell walls in the root cortex (up to 90%; Mengel and Kirkby, 1987; Sieghardt, 1990; Turner, 1970). However, the amount of total Zn in the roots may be a function of the duration of exposure. There is evidence that the binding sites in roots for some metals, such as Pb, must become saturated before they are translocated to the shoot tissue (Dushenkov et al., 1995; Kumar et al., 1995). Zinc is also bound to vascular tissue in roots and stems (Longnecker and Robson, 1993; Riceman and Jones, 1958), with the majority of Zn being found where vascular tissue traces off the central stele at the nodes or in regions of the root system where lateral roots are present (Singh and Steenberg, 1974). Under adequate supply, the Zn absorbed by roots is rapidly transported to the shoots (Riceman and Jones, 1958). Movement in the plant is not necessarily via passive transport in the transpirational stream, as there is evidence that accumulation occurs in parts of the plant were transpiration is minimal, not where the greatest transpiration is occurring (Obata and Kitagishi, 1980). There is little remobilization of Zn throughout the plant, particularly when present at deficient or adequate levels (Longnecker and Robson, 1993). When the supply of Zn to *Trifolium subterraneum* is low to adequate (0 to 0.13 mg · kg^{-1} soil) the concentration in the younger leaves is usually higher than that of older leaves. However, when supply is high (0.53 to 2.13 mg · kg^{-1} soil), Zn accumulates in older leaves of plants, and the concentration in older leaves is much higher than that in new growth (Longnecker and Robson, 1993; Reuter et al., 1982; Ruano et al., 1987).

Summary of Literature Review

The pollution of soil and water with heavy metals is an environmental concern today. Metals and other inorganic contaminants are among the most prevalent forms of contamination found at waste sites, and their remediation in soils and sediments are among the most technically difficult. The projected cost for remediation of areas containing mixtures of heavy metals and organic pollutants by conventional means is $35.4 billion over the next five years. The high cost of existing cleanup technologies led to the search for new cleanup technologies that have the potential to be low-cost, low-impact, visually benign, and environmentally sound. Phytoremediation is a new cleanup concept that involves the use of plants to clean or stabilize contaminated environments. The most studied phytoremediation technology is phytoextraction, a plant-based cleanup method involving the use of metal-accumulating plants to extract metal contaminants from soil. Once metals have been sequestered in the tissues, the above-ground portions of the plant can be harvested resulting in the permanent removal of metals from the site. Phytoextraction is not the answer to all environmental problems, but rather is another tool to be used in conjunction with existing remediation technologies. However, in areas that have been contaminated with low to moderate levels of heavy metals at shallow depth, phytoextraction has some advantages over conventional cleanup methods, the primary one being low cost.

Over the past decade, researchers have sought to perfect this remediation technology. The majority of phytoextraction research has focused on finding the ideal metal-accumulating plant and the means by which metals can be liberated from the soil for root uptake. At present, Indian mustard (*B. juncea*) is among the most viable candidates for the phytoextraction of a number of metals including Cd, Cr(IV), ^{137}Cs, Cu, Ni, Pb, U, and Zn. Only a fraction of the phytoremediation research addresses the use of *B. juncea* for the phytoextraction of Zn even though this element is one of the most prevalent heavy metals at contaminated sites. Zinc is among the least toxic of all metal contaminants to humans, and this may be the reason why other metals have been given priority in phytoremediation research efforts in the past. Few studies have focused on the development of specialized agricultural practices for phytoextraction, although most researchers agree that this is an area that warrants further attention. Mineral nutrition profoundly influences the growth of plants and the absorption of nutrients by plant roots, two areas with which phytoextraction is greatly concerned. With the exception of Zaurov et al. (1999), all plant nutrition information for *B. juncea* with regards to phytoremediation has been anecdotal (Ebbs et al., 1997; Ebbs and Kochian, 1997, 1998; Kumar et al., 1995). Proper plant nutrition has the potential to be an effective, low-cost agronomic practice for enhancing the phytoextraction of heavy metals by plants, but more research is required before fertilizers can be used effectively for this purpose.

Table 1. Costs associated with various types of remediation methods.

Type of Medium	Remediation Method	Range of remediation cost (in dollars) soil = per cubic meter water = per 1000 gallons cleaned	
Soil	In Situ Vitrification†, ‡	360	1,370
bulk density=1.3	Soil Incineration ‡	200	1,500
	Excavation and Landfill‡, §, ¶, #	140	720
	Soil Washing†, ‡, ††, ‡‡	80	860
	Soil Flushing†, ‡	50	270
	Solidification and Stabilization †	40	200
	Electrokinetic Systems†, ‡	30	290
	Bioremediation†	10	310
	Phytoremediation of Soil‡, §, ¶, ‡‡	<1	150
Water	Activated Carbon ‡	120	210
	Biosorption ‡	9	3,400
	Reverse Osmosis‡	3	3
	Adsorption ‡	1	20
	Membrane separation-filtration ‡	1	6
	Rhizofiltration ‡, ¶	<1	6
	Ion Exchange‡	<1	2
	Chemical Precipitation ‡	<1	2

† Woods, 1997
‡ Glass, 2000
§ Salt et al., 1995a
¶ Cunningham et al., 1997
¶ Ensley, 2000
†† Dennis et al., 1994
‡‡ Black, 1995

Note: Reported costs are estimates from available data. All soils were assigned a bulk density of 1.3 for the purposes of comparison.

Table 2. Massachusetts DEP standards for various inorganic contaminants in soil.

Contaminant	Concentration Limit (ppm) for Contaminants in Soil†	
	High Exposure Potential‡	**Low Exposure Potential**
Arsenic	30	30
Cadmium	30	80
Chromium III	1000	5,000
Chromium VI	200	1,000
Cyanide	100	400
Fluorine	400	5000
Lead	30	600
Mercury	20	60
Nickel	300	700
Selenium	400	2,500
Zinc	2,500	5,000

† All concentrations in soil are presented on a dry weight basis.
‡ High exposure=S-1 (Method 1); Low exposure=S-3 (Method-1); Exposure level is determined by the frequency of visitation, the intensity of site activities, the accessibility of the contamination, and the age of those potentially at risk for exposure. See reference for further details.

(MADEP) Massachusetts Department of Environmental Protection Publication. 1993. 310 CMR 40.0000: Massachusetts Contingency Plan (MCP).

Table 3. Promising plants for the phytoextraction of various metals and radionuclides.

Metal or Radionuclide	Plant Species (reference indicated by typographical symbols)
Cd	*Brassica juncea* (L.) Czern†
Cr (VI)	*Brassica juncea* (L.) Czern†
^{137}Cs	*Amaranthus retroflexus* L.‡; *Brassica juncea* (L.) Czern.‡, §; *Brassica oleracea* L.§; *Phalaris arundinacea* L.§; *Phaseolus acutifolius* A.Gray.‡, §
Cu	*Brassica juncea* (L.) Czern†
Ni	*Brassica juncea* (L.) Czern†
Pb	*Brassica campetris* L.†; *Brassica carinata* A. Br.†; *Brassica juncea* (L.) Czern.†, ¶, #, ††; *Brassica napus* L.†; *Brassica nigra* (L.) Koch.†; *Helianthus annuus* L.†; *Pisum sativum* L.‡‡; *Zea mays* L.‡‡
Se	*Brassica napus* L.§§; *Festuca arundianacea* Schreb.§§; *Hibiscus cannabinus* L.§§
U	*Brassica chinensis* L.¶¶; *Brassica juncea* (L.) Czern.¶¶; *Brassica narinosa* L.¶¶
Zn	*Avena sativa* ##; *Brassica juncea* (L.) Czern.†, ††, ##, †††; *Brassica napus* L.†††; *Brassica rapa* L.†††; *Hordeum vulgare*##

† Kumar et al., 1995
‡ Lasat et al., 1998
§ Lasat et al., 1997
¶ Begonia et al., 1998
# Blaylock et al., 1997
†† Salt et al., 1995b
‡‡ Huang et al., 1997a
§§ Banuelos et al., 1997
¶¶ Huang et al., 1998
Ebbs and Kochian, 1998
††† Ebbs and Kochian, 1997

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