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LITERATURE REVIEW:
PHYTOACCUMULATION OF CHROMIUM, URANIUM, AND PLUTONIUM IN PLANT SYSTEMS

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INTRODUCTION

Phytoremediation: A General Review

Phytoremediation is an integrated multidisciplinary approach to the cleanup of contaminated soils, which combines the disciplines of plant physiology, soil chemistry, and soil microbiology. Metal hyperaccumulator plants are attracting increasing attention because of their potential application in decontamination of metal-polluted soils. Traditional engineering technologies may be too expensive for the remediation of most sites. Removal of metals from these soils using accumulator plants is the goal of phytoremediation (Baker et al., 1994; Brown et al., 1994; Brown et al., 1995a; Brown et al., 1995b; Blaylock et al., 1997; Carey, 1996; Chaney et al., 1997; Cunningham and Ow, 1996; Cunningham et al., 1996; Dushenkov et al., 1995; Moffat, 1995; Nanda Kumar et al., 1995; Raskin et al., 1994; Rouhi, 1997; Salt et al., 1995). The emphasis of this review has been placed on chromium (Cr), plutonium (Pu), and uranium (U). With the exception of Cr, these metals and their decay products exhibit two problems, specifically, radiation dose hazards and their chemical toxicity. The radiation hazard introduces the need for special precautions in reclamation beyond that associated with non-radioactive metals.

The uptake of beneficial metals by plants occurs predominantly by way of channels, pores, and transporters in the root plasma membrane. Plants characteristically exhibit a remarkable capacity to absorb what they need and exclude what they don't need. But most vascular plants absorb toxic and heavy metals through their roots to some extent, though to varying degrees, from negligible to substantial. Sometimes absorption occurs because of the chemical similarity between beneficial and toxic metals. Some plants utilize exclusion mechanisms, where there is a reduced uptake by the roots or a restricted transport of the metal from root to shoot. At the other extreme, hyperaccumulator plants absorb and concentrate metals in both roots and shoots (Baker, 1981). Some plant species endemic to metalliferrous soils accumulate metals in percent concentrations in the leaf dry matter (Brooks et al., 1977).

The term 'hyperaccumulator' was introduced by Brooks et al. (1977) for plants growing on serpentine sites that are capable of concentrating nickel (Ni) to more than 1000 $\mu g\ g^{-1}$ (0.1 percent) in their leaves on a dry matter basis. A concentration of 1000 $\mu g\ g^{-1}$ has also been used to delineate exceptional uptake of copper (Cu), cobalt (Co), and lead (Pb). The delineation level is raised to 10,000 $\mu g\ g^{-1}$ (1.0 percent) for zinc (Zn) and manganese (Mn) because of greater background concentrations of these metals in soil.

Chaney et al., (1995) proposed that a viable phytoremediation technology will require breeding improved cultivars of hyperaccumulators and development of improved agronomic practices, or for species like *Thlaspi*, for which yield is too low to support phytoremediation, bioengineering may be necessary to develop high biomass hyperaccumulating plants. They concluded that hyperaccumulator plants could be developed to remediate soils contaminated with heavy metals, and that before the year 2000, commercial phytoremediation will compete with engineering approaches for remediation of contaminated soils. Entry et al. (1996) proposed that the concentration of radionuclides in the ash of incinerated hyperaccumulators is a more desirable outcome than the mechanical methods currently employed.

Phytoremediation requires that the target metal must be (1) available to the plant root, (2) absorbed by the roots, and (3) translocated from the root to the shoot. The metal is removed from the site by harvesting the plant material. After harvesting, the biomass is processed to either recover the metal or further concentrate the metal to facilitate disposal.

The concept of using hyperaccumulator plants to decontaminate industrially-contaminated soil was first tested at a farm managed by Rothamsted Experiment Station in England, in which the field was polluted with zinc (McGrath et al., 1993). Ten plant species were tested over four seasons. These were: *Thlaspi careulescens*, *Thlaspi achroleucum*, *Cadominopsis halleri*, *Reynoutria sachalinense*, *Cochlearia pyrenaica*, *Alyssum lesbiacum*, *Alyssum murale*, *Raphanus sativus* (radish), and *Brassica napus* (spring rape). The greatest Zn uptake was obtained with *Thlaspi careulescens*, which had the potential to remove the equivalent of over 40 kg Zn ha^{-1} yr^{-1}. Baker et al. (1991) conducted a pot study with soils from long-term field plots, using metal-tolerant (including hyperaccumulators) and normal plants. They concluded that phytoremediation, using certain species, could offer a low cost, low technology alternative to current clean up technologies. Ernst (1988) harvested plants from natural stands on several contaminated sites and came to a different conclusion. He measured the relative abundance and metal uptake of various plant species and concluded that phytoremediation was not a viable remediation technology. Although hyperaccumulator plants were present on contaminated sites, they were not harvested because of their low growing, rosette characteristics. Ernst (1988) concluded that it would be impractical to harvest rosette-type plants with mechanical equipment. New technologies would need to be developed to make harvesting of plants with these growth habits feasible.

Hyperaccumulation is often associated with plants with relatively slow growth rates. Some plant species have lower shoot concentrations of metals but greater biomass production. For example, the natural growth pattern of *Thlaspi* is problematic for mechanical harvesting. But

Silene, which accumulates metals less than *Thlaspi*, grows more rapidly and vigorously. *Silene* is also more capable of colonizing a contaminated site because of its seed and rhizome production. These factors would favor the establishment and harvesting of *Silene* over *Thlaspi* (Baker et al., 1994).

Fundamental to the environmental and economic success of phytoremediation is the existence of plant genotypes which hyperaccumulate metals. To maximize the metal concentration in the biomass, it will be necessary to use a combination of improved soil management inputs (e.g., optimized soil pH and mineral nutrition, minimum concentrations of interfering elements, and the introduction of agents which increase the concentration and diffusion of metals in the soil), improved genotypes with optimized metal uptake, translocation and tolerance, and improved biomass yield (e.g., > 20 t ha^{-1}). Individualized practices may need to be developed for specific sites.

This report focuses on previous, current and proposed research concerning the interactions of Cr, Pu and U with plants in relation to the development of better practices for phytoremediation.

Distribution of Hyperaccumulator Plants

Hyperaccumulator plants are geographically distributed and are found throughout the plant kingdom. The approximately 400 taxa shown in Table 1 include representatives of many families, ranging in growth habit from annual herbs to perennial shrubs and trees. Hyperaccumulator plants have been identified on all continents, both in temperate and tropical environments. Natural occurrences of hyperaccumulators for Ni include New Caledonia, Cuba, Southeast Asia, Brazil, southern Europe and Asia Minor; for Zn and Pb include northwest Europe; and for Cu and Co include south-central Africa. Some families and genera are particularly well documented for Ni [*Brassicaceae* (*Alyssum* and *Thlaspi*), *Euphorbiaceae* (*Phyllanthus*, *Leucocroton*) and *Asterceae* (*Seeio*, *Pentacalia*)], Zn *Brassicaceae* (*Thlaspi*), and Cu and Co (*Lamiaceae*, *Scrophulariaceae*) (Baker et al., 1991; Baker and Brooks, 1989) (Table 1).

There are not many Cr hyperaccumulators in nature, but there are numerous Ni hyperaccumulators (Table 1). Few Cr hyperaccumulators have been identified, partly because in nature Cr exists predominantly in the +3 oxidation state and is very insoluble and much less available for plant uptake.

Multi-Metal Accumulators

The ability of a plant to hyperaccumulate any one metal may infer some ability to accumulate other metals (Reeves and Baker, 1984; Baker et al., 1994). Some metals may interact competitively for accumulation (e.g., Zn and Ni in calamine and serpentine soils). The number of Ni hyperaccumulator taxa are more than 300 in 35 families. They commonly have 3-4% Ni in leaves dry matter but may range to as high as 25%.

Alyssum betolonii, which is endemic to serpentine soils, is known for its high concentration of Ni (> 10,000 mg kg^{-1} in leaves). The fact that serpentine (ultramafic) soils also contain other elements such as Cr has led to the assumption that the preferential accumulation of Ni in many species of *Alyssum* is due to a selective uptake mechanism. Gabbrielli et al., (1991)

TABLE 1

Numbers of Metal Hyperaccumulators Plants Based on All Records Available as of March 1995

Metal	Concentration (% in leaf dry matter)	No. of taxa	No. of families
Cd	>0.01	1	1
Co	>0.1	26	12
Cu	>0.1	24	11
Pb	>0.1	5	3
Ni	>0.1	>300	35
Mn	>1.0	8	5
Zn	>1.0	18	5

showed, in controlled experiments, that excised roots of *Alyssum bertolonii* seedlings did not show selectivity for uptake of a specific metal. The plant roots tend to accumulate Ni, Co and Zn, without discriminating between them, and with the same saturation trend, demonstrating the absence of a competitive action between these three elements. Clones of *Salix viminalis* were also found with high concentrations of heavy metals (Cd, Cu and Zn) in their shoots.

Baker et al. (1994) suggested common mechanisms of absorption and transport of several metals by *Thlaspi* species. They observed high uptake by the roots for all metals studied. Zinc, cadmium (Cd), Co, Mn and Ni were readily transported to the shoot, whereas, aluminum (Al), Cr, Cu, iron (Fe) and Pb were predominantly immobilized in the roots.

Reeves and Baker (1984) showed that hyperaccumulator plants growing naturally in calcareous soils are able to tolerate serpentine soil and absorb elements other than Ni. It was observed when a population of *Thlaspi goesingense* 'Halacsy' taken from calcareous soil was grown on a serpentine soil, extremely high concentrations of Ni, Zn, Co, and Mn were accumulated in the above-ground dry matter. The absorbed metal concentrations were similar to those observed with *Thlaspi* growing naturally in serpentine soils.

Potential Accumulators with High Biomass Production

Indian mustard (*Brassica juncea*) is a high biomass, rapidly growing plant that has an ability to accumulate Ni and Cd in its shoots. It is a promising plant for phytoremediation and is suitable for genetic transformation (Terry et al., 1992).

Huang, et.al. (1976b) investigated phytoextraction of Pb from contaminated soils. They used two Zn hyperaccumulators and an Fe accumulating pea mutant (E107). Zinc hyperaccumulators accumulated Pb mainly in their roots while the pea mutant accumulated Pb in its shoots. They suggested that the use of plant mutants may assist in the understanding of processes involved in Pb hyperaccumulation as well as in the development of Pb hyperaccumulators. They also found that foliar phosphate application increased shoot dry weight 5 fold and increased total Pb accumulated in shoots by 115 percent, since soil applied phosphate fertilizer binds Pb and decreases its solubility in the soil.

Dushenkov et al. (1995) observed that roots of many hydroponically grown terrestrial plants such as Indian mustard (*Brassica juncea* (L.) Czem) and sunflower (*Helianthus annuus* L.)

effectively removed the potentially toxic metals, Cu, Cd, Cr, Ni, Pb and Zn, from aqueous solutions.

Mechanisms for Metal Tolerance

Plant species that are naturally high in heavy metals have developed a strategy to tolerate the heavy metals by unrestricted absorption and, as a result, accumulate high concentrations of the heavy metal in the plant tissue. Since heavy metals are damaging to most plants at relatively low concentrations, the hyperaccumulation strategy requires some mechanism(s) to detoxify the metals.

It is widely accepted that detoxification of metal ions within plant tissues must depend on chelation by appropriate ligands. There has been considerable interest in determining the chemical nature of these ligands. The anionic species of organic acids, such as citrate, malate, and malonate, are commonly found in high concentrations in the leaves of *Alyssum* spp. Reeves (1992) has pointed out that these anions tend to be present constitutively in these plants in substantial amounts and cannot account for the metal-specificity or species variability of Ni hyperaccumulators. Andrew et al. (1995) suggested that the Ni hyperaccumulation trait in *Alyssum* is was associated with the ability of the root system to produce substantial amounts of histidine as a Ni complexing ligand.

Streptanthus polygaloides 'Gray' (Brassicaceae) is a Ni hyperaccumulator native to serpentine soils in northern California. This species tolerates 500 µM Ni without developing toxicity symptoms, and actually grows better with Ni than without Ni. It accumulates Ni in both roots and shoots to concentrations as high as 2 percent on a dry weight basis. One function of Ni hyperaccumulation in *S. polygaloides* is as a defense against pathogens (Boyd et al., 1994). Their preliminary data has demonstrated that at least two Ni-binding proteins are unique to plants grown with Ni.

Metals are required for a variety of metabolic processes in all organisms. However, because many metals can be toxic, plants have evolved systems to regulate the uptake and distribution of metals. For plants, uptake of metals occurs primarily through the roots, so this is the primary site for regulating their accumulation. Once metals have crossed the root membrane, there are a variety of mechanisms to prevent metal toxicity, including compartmentation and binding to intracellular ligands. In addition to the organic acids, plants are equipped with at least two ligands that are able to bind heavy metals such as Cu, Zn and Cd: phytochelatins (PCs) and metallothioneins (MTs).

Phytochelatins are a family of peptides with the general structure $[\bullet\text{-GluCys}]_n\text{-Gly}$, where $n>1$ (Rauser, 1990). Metallothioneins (MTs) are similar to PCs in being Cys-rich, metal-complexing ligands. Metallothioneins, however, are proteins synthesized by mRNA translation.

CADMIUM

Cadmium (Cd) Uptake and Translocation

The amount of Cd accumulated within the plant is limited by several factors, including: (1) Cd bioavailability within the rhizosphere, (2) rates of Cd transport into roots via either the apoplastic or symplastic pathways, (3) the proportion of Cd fixed within roots, as a Cd-phytochelatin complex, and accumulated within the vacuole, and (4) rates of xylem loading and translocation of Cd.

Cadmium bioavailability depends on soil pH, redox potential, and rhizosphere chemistry. These factors determine the concentration of soluble Cd within the rhizosphere and the amount of Cd available for potential uptake by the plant. Soluble Cd could enter roots either by movement in the cell wall free space (apoplastic pathway) or by transport across the plasma membrane (PM) of root cells and movement through the cytoplasm (symplastic pathway). The large membrane potential which usually exists across the PM provides a driving force for the inward movement of Cd into cells; however, for this to occur, some type of channel must exist within the PM which allows Cd transport.

Chemical detoxification of heavy metals within the plant cell may be achieved by (1) binding or sequestration by metal-complexing agents, (2) transport of the heavy metals to cellular compartments (i.e., vacuole), or (3) a combination of both mechanisms. It has been proposed that, in the case of Cd, PCs are involved in differential metal tolerance. Increased tolerance might be accomplished by a more rapid formation of stable PC-metal complexes due to: (1) an increased production of PCs, (2) a higher amount of longer chain forms of PC, or (3) increased incorporation of sulfur-containing amino acids.

Cadmium entering root cells can be sequestered within the vacuole, as a Cd-phytochelatin complex, where it is unavailable for translocation to the shoot. However, it may also travel across the cell and ultimately enter the translocation stream. Cadmium transport into the vacuole can either occur via a Cd^{2+}/H^+ antiport or as a Cd-phytochelatin complex energized by the direct hydrolysis of ATP. Uncomplexed phytochelatins can also be transported into the vacuole in an ATP-dependent manner.

For transport to the leaves, Cd must first pass into the endodermis before it can enter the xylem vessels. The endodermis is surrounded by a strip of subrinized cell wall, called the casparian strip, which inhibits apoplastic transport. Therefore, before Cd can enter the endodermis, and hence the xylem vessels, it must first move symplastically to avoid the casparian strip. Cadmium appears to be translocated within the xylem chelated by oxygen atoms, probably by the conjugate bases of organic acids (Salt and Raskin, 1995).

Gene Regulation by Cadmium

Specific enzymatic and regulatory roles have been assigned for most genes of the anthocyanin pathway as a result of genetic and biochemical analysis. At least one of these genes, with homology in several plant species, is regulated by heavy metals, particularly Cd. Anthocyanins are located in the vacuole and share a common synthetic origin and a core structure with species-specific decoration of the core by hydroxylation, methylation, sugar addition or acylation (Stafford, 1990). These modifications as well as acidification in the vacuole result in the diverse red, blue, and purple colors observed in flowers, fruits, stems and leaves.

In maize (Marrs et al. 1996), the bronze 2 gene (*Bz2*) encodes for the enzyme, glutathione S-transferase (GST) as does the soybean counterpart gene *GmGST26-A* (Ulmasov et al. 1995). This enzyme, located in the cytosol (Ulmasov et al. 1995), performs the last genetically defined step in anthocyanin biosynthesis, namely tagging an anthocyanin precursor, cyandin-3-glycoside with glutathione, allowing for recognition and targeting of anthocyanins into the vacuole via and Mg/ATP-dependent, ABC-type GSH pump located in the tonoplast (Marrs, et al, 1996). This mechanism has been confirmed by the use of vanadate, which inhibits transport into the vacuole by inhibiting the tonoplast GSH pump (Marrs et al. 1996). Both *Bz2* and *GmGST26-A* belong to a group of type III *GST*s, which are induced by a variety of environmental stresses, including heavy metals such as Cd (Marrs and Walbot, 1997). Cadmium induces a higher transcription rate of Bz2, and apparently two, not one, species of mRNA are produced. In addition to one mRNA species coding for GST, a second mRNA species codes for a new truncated GST protein which is missing the enzyme activity domain, but retains the dimerization and glutathione (GSH)-binding domains. Therefore, the end result is two proteins, one of which is the normal GST which targets the anthocyanin precursor to the vacuole, while the other appears to be involved in the heavy metal transport into and accumulation within the vacuole (Marrs and Walbot, 1997).

Cadmium Accumulation in Vacuoles

In addition to induction of GST production, heavy metals also induce the synthesis of phytochelatins (PCS), which are proteins embodied with GSH peptides and are known to complex with heavy metals (Rauser, 1995). For example, Cd, has been shown to induce the biosynthesis of both GSTs and PCS (Rauser and Meuwly, 1995). Marrs and Walbot (1997) hypothesize that the newly synthesized, truncated GST induced by Cd, fuses with the PC-heavy-metal complex and shuttles it across the tonoplast and into the vacuole. In soybean *GmGST26-A*, a gene induced by Cd, also produces both the full length and the truncated GST proteins (Czarnecka et al. 1988). Therefore, these genes appear to encode multifunctional stress proteins which may be critical in heavy metal detoxification. Studies with the promoter sequence of *GmGST26-A* fused to the reporter gene, *uidA*, show that the promoter is induced by Cd and a variety of other chemical agents including hydrogen peroxide (Ulmasov et al., 1995). Ulmasov et al. (1995) theorized that GSTs are synthesized in response to an oxidative stress, i.e., a general stress that is the result of a variety of agents such as heavy metals that cause the production of active oxygen species. Droog et al., (1993) have proposed that heavy metals cause oxidative damage to plant cells which subsequently results in the synthesis of GSTs. Thus, *Bz2* and *GmGST26-A* may not be activated by a signal transduction pathway specific to heavy metals, but are most likely activated by a pathway mediated by oxidative stress (possibly an active oxygen species).

CHROMIUM

At the early stage of the current project, we proposed to use Cr as an analog for plutonium (Pu) and uranium (U). Our interest in Cr resulted from the analogous oxidation behavior of Cr, U, and Pu, i.e., readily hydrolyzed and relatively insoluble at the lower oxidation states in which the predominant species are cationic (Cr^{3+} and its hydrolysis species) and more soluble and more mobile at the higher oxidation states (Cr^{6+}) in which the predominant species are anionic, e.g., $CrO_4{}^{2-}$. Chromium, U, and Pu have different atomic radii, but each has a strong tendency to bind to oxygen-containing functional groups, including simple organic complexing agents such as oxalate and citrate (Garland et al., 1983). Like Pu and U, Cr is mainly accumulated on or in the roots (Shewry and Peterson, 1974; Cary et al., 1977a). It is conceivable that Cr hyperaccumulator plants might also have the ability to hyperaccumulate Pu or U.

Chemistry of Soil Chromium

Occurrence of Chromium. Chromium is widespread in the environment, with small amounts usually being present in rocks and soils as chromic oxides. Total Cr in igneous and sedimentary rocks commonly ranges from 10 to 100 mg kg^{-1}. Soils high in Cr(III) are found naturally where the parent materials are derived from serpentine rocks. There are occurrences of serpentine-rich soils in North America (California, Pennsylvania, Maryland) that contain several percent Cr.

The use of Cr in chrome plating, paints, corrosion inhibitors, reinforced steel, and fungicides all contribute to its dissemination in the environment. Numerous sites in the U.S. are contaminated with Cr (Palmer and Wittbrodt, 1991). Soils of the United States commonly contain from 1 to 1,500 mg Cr kg^{-1}. The permissible limits are 50 µg Cr L^{-1} in potable water and 1.7 mg L^{-1} in effluent water. Chromium(VI) is generally considered to pose the greatest human health risk because it is more toxic, more soluble, and more mobile than Cr(III) (James, 1996).

Forms of Soil Chromium. A number of soil processes and factors may affect the form and biomobilization potential of Cr (Allaway, 1975; Otabbong, 1990). Chromium exists predominantly in the +3 and +6 oxidation states. The intermediate states of +4 and +5 are metastable and rarely encountered.

Chromium (III) is largely present in soil as relatively unavailable, insoluble oxides of Cr and Cr-Fe. It can also exist as a substitute for Al(III) in the $[AlO_6]$ groups of aluminosilicates. The solubility of Cr(III) in soil is dependent on pH (Palmer and Wittbrodt, 1991) and decreases dramatically at pH > 4.5. At pH values less than 3, the predominant form of Cr in the Cr(III)-H_2O system is Cr(III). With increasing pH, Cr(III) is hydrolyzed. The most important species are $CrOH^{2+}$, $[Cr(OH)_3]^{\circ}$, and $[Cr(OH)_4]^-$, with $[Cr(OH)_2]^-$ occurring in a significant proportion only in the narrow pH range between 6.27 and 6.84. Chromium(III) can form stable complexes with organic ligands, including oxalate, citrate, malate, EDTA and DTPA, and polymers, at pH values as high as 8.5. These complexes form slowly, and once formed, are difficult to break. The presence of organic complexes can significantly influence the concentration of total dissolved Cr(III) in the soil solution.

Chromium(VI) is considerably more soluble than Cr(III). Chromate, CrO_4^{2-}, which is the predominant form at pH>6, exists in pH-dependent equilibrium with other forms of Cr(VI) such as $HCrO_4^-$ and dichromate ($Cr_2O_7^{2-}$). Chromium (VI) can be adsorbed by positively-charged soil components such as Al and Fe oxides (Palmer and Wittbrodt, 1991), as well as at positive charged sites at the surface of plant roots. Adsorption of Cr(VI) is considerably less at neutral to alkaline pH than at more acidic pH values (Bartlett and Kimble, 1976a,b; Bartlett and James, 1983). The intensity of adsorption will depend on the type and quantity of soil components, as well as pH and the presence of competing ligands such as phosphate.

The availability of soil Cr to the plant depends on the oxidation state of Cr, pH, and the presence of colloidal binding sites and Cr-organic complexes that would influence its total solubility.

Oxidation/Reduction of Chromium Species. Chromium(VI) is not thermodynamically stable in soils except in alkaline, oxidizing environments, and is readily reduced to Cr(III) (Cary et al., 1977b). The transformation of Cr(VI) to Cr(III) in soils is likely to occur as a result of reduction by Fe(II) in solution and at mineral surfaces, reduced sulfur compounds, or soil organic matter (Bartlett, 1991; Charlet and Manceau, 1992; Fendorf, 1995; James and Bartlett, 1983b; Losi et al., 1994). The reduction of Cr(VI) to Cr(III) by organic matter is more rapid in acid than in alkaline soils (Cary et al., 1977b). Because Cr(VI) can be so easily reduced to Cr(III), under most soils conditions, Cr(III) can be expected to be the predominant form (James and Bartlett, 1983c).

Although oxidation of Cr(III) to Cr(VI) is possible, especially in the presence of manganese oxides (Palmer and Wittbrodt, 1991), oxidation usually only occurs under moist conditions, and not appreciably in dry soils (Bartlett and James, 1979). Soil Cr is sometimes mobilized when soils are flooded and then drained or incubated with organic matter, presumably by the oxidation to Cr(VI) or association of Cr(III) with soluble organic complexing agents. In natural systems, manganese oxides are the only compounds capable of oxidizing Cr(III) to Cr(VI) (Fendorf, 1995).

Role of Chromium in Plants and Animals

Chromium is essential to humans and animals for glucose metabolism. Chromium (III) is bound almost entirely to a siderophilin protein at unfilled Fe-bonding sites. There is no evidence that Cr has any physiological function in plants. Although chromium is not considered to be essential for plant growth and development, some studies have indicated that at low concentrations (1 µM), Cr stimulates plant growth (Bonet et al., 1991). Chromium is toxic for agronomic plants at about 0.5 to 5.0 $\mu g\ mL^{-1}$ in nutrient solution and 5 to 100 $\mu g\ g^{-1}$ of available Cr in soil.

Plant Uptake of Chromium

Under normal conditions, the concentration of Cr in the plant is $< 1\ \mu g\ g^{-1}$. Chromium uptake by plants is mainly non-specific, probably as a result of plant uptake of essential nutrients and water. Plants can absorb both Cr(VI) and Cr(III). Chromium(VI) is more toxic than Cr(III) and is more easily transported inside the plant (Mortvedt and Giordano, 1975). Chromium(VI) uptake has been reported to occur by an active mechanism, whereas Cr(III) uptake is passive, indicating that the two forms do not share a common uptake mechanism (Skeffington et al., 1976). Once in the xylem, CrO_4^{2-} is more easily transported than Cr(III), presumably because the latter is bound by ion exchange on the vessel walls or precipitated as $Cr(OH)_3$. Cr-EDTA moves faster than Cr(III) ions to the shoots, due to the higher solubility of the former in aqueous solution. Cr3+ is predominantly transported in the form of organic complexes. For example, the only species of Cr(III) identified in xylem sap of *Leptospernum scoperium* following the addition of $CrCl_3$ was the Cr(III) trioxalate complex.

Many studies have reported that plants absorb Cr(VI) better than Cr(III), based solely on plant concentration data and the observation that Cr(VI) is more toxic to plants than Cr(III) (Barcelo *et al.*, 1986; Hara and Sonoda, 1979; Lee et al., 1981; Peterson and Girling, 1981). For example, Cr(VI) was reported to be more toxic to the growth of barley in solution culture than Cr(III) supplied as $CrCl_3$ (Skeffington et al., 1976). A problem of any study of this type is that the solubilities of Cr(VI) and Cr(III) are not comparable under the same conditions (McGrath, 1982). In a study of Cr uptake by oat, McGrath (1982) used a flowing culture technique in which equal concentrations of soluble Cr(VI) and Cr(III) were maintained, and found that the plants absorbed Cr(VI) and Cr(III) equally well. Using metabolic inhibitors, Shewry and Peterson (1974) and Skeffington et al. (1976) demonstrated that plant uptake of Cr(VI) was an active process, while uptake of Cr(III) was a passive process, i.e., no energy expenditure by the plant was required. If this is the case, then it is logical to conclude that plants should be able to continuously absorb Cr(III) if it is soluble in the medium.

Plant species differ significantly in Cr uptake capacity and distribution within the plant (Grubinger et al., 1994; Soane and Saunder, 1959). In an attempt to find approaches to increase

Cr contents of food crops, Cary et al. (1977a) compared Cr uptake by various dicotyledonous and monocotyledonous food crops. They found that, in general, dicots such as buckwheat and rutabaga absorbed more Cr and transported more Cr to shoots than did monocots such as corn and barley. The reason for this difference between dicots and monocots has not been definitely determined; however, the differences in rooting pattern, transpiration rate and metabolism between the two groups of plants could be among the reasons. Their generally greater uptake of Cr suggests that dicots might be reasonable target species for Cr hyperaccumulation. Several of the previously identified Cr hyperaccumulator plants are dicots. Although most plants are not able to hyperaccumulate Cr, a few plant species have been identified as Cr hyperaccumulators, when grown on high Cr soils. Wild (1974) has reported extraordinarily high concentrations of Cr in leaves of *Dicoma niccolifera* (1,500 µg g^{-1} dry weight) and *Sutera fodina* (2,400 µg g^{-1}) (Baker and Brooks, 1989) (both species were located in Zimbabwe), while *Lepertospermum scoparium* from an abandoned chromite mine in New Zealand contained up to 1 percent Cr (Lyon et al., 1971). Another plant species from Zimbabwe, *Pearsonia metallifera*, was also reported to contain high concentrations of Cr (Wild, 1974).

Phytotoxicity of Chromium

Chromium is potentially toxic to higher plants at total tissue concentrations of • 0.1 mmol kg^{-1} dry weight (Mengel and Kirkby, 1982). Chromium has a direct toxic effect on roots and a direct or indirect effect on leaves. Mukherji and Roy (1978) concluded that Cr(VI) seems to act principally on plant roots, resulting in intense growth inhibition. The initial symptoms of Cr toxicity appeared as severe wilting and chlorosis of plants (Turner and Rust, 1971).

Chlorosis, which appears in the upper leaves of Cr-affected plants, has been proposed as an indirect effect of Cr, probably due to the retardation of Fe and Zn translocation. The primary toxic effect seemed to be membrane damage due to the high oxidative potential of Cr(VI).

Chromium toxicity can occur when soil is contaminated with Cr(VI) and the pH is high or when soil is contaminated with Cr(III) or Cr(VI) and the pH is low. In the latter case, Cr(VI) will be reduced to Cr(III), which will then equilibrate with the soil solution. Chromium is precipitated at higher pH values as the Cr(III) hydroxide and is unavailable to plants.

Plant Uptake and Transformation. Trivalent and hexavalent Cr were both absorbed by oat and were equally toxic when supplied at 2 to 200 μM concentrations in nutrient solution. The proportion of Cr translocated to the shoots was similar, regardless of the form supplied to roots. The addition of 750 μg Cr(VI) g^{-1} of either acid or alkaline soil resulted in high, toxic concentrations of Cr in the soil solution. The same amount of Cr(III) gave a lower concentration in the soil solution due to precipitation, with higher soil solution Cr present in acid compared to alkaline soils (McGrath, 1982). The low solubility of Cr(III) coupled with its strong retention on soil surfaces limits its bioavailability and mobility in soils and waters (Bartlett, 1988). Although Cr(III) is not a significant hazard in itself, the potential for oxidation to Cr(VI) can make its risk tantamount to that of the hexavalent form.

Plants must have the ability to translocate Cr from the root to the shoot, or to compartmentalize it, in order for the plant to continue absorption of Cr from the medium, since a high concentration of Cr is toxic to plants. There are at least two advantages to the plant for translocation: (1) it can reduce Cr concentration and thus reduce toxicity potential to the root, and (2) translocation to the shoot is one of the mechanisms of resistance to high Cr, because for some plants the high concentration of Cr will be lost when leaves fall in the autumn. Most research using non-hyperaccumulator plants has shown that Cr is mainly accumulated in the roots, and much less of the total Cr in a plant is in the leaves (Shewry and Peterson, 1974; Cary et al., 1977a). The distribution of Cr between root and shoot in hyperaccumulator plants, however, indicated that the leaves also contained a much higher Cr concentration than that of non-hyperaccumulator plants, suggesting better translocation of Cr from root to shoot for hyperaccumulator plants. Although the detailed mechanisms of Cr translocation are not understood, there are reports that Fe-deficient and P-deficient plants can better translocate Cr from roots to shoots (Cary et al., 1977a; Bonet et al., 1991). These results lead to the hypothesis that Fe- and P-deficiency induced accumulation of organic acids, e.g., citric acid (Landsberg, 1981; De Vos et al., 1986; Johnson et al., 1994), may play an important role in Cr translocation. Using a hyperaccumulator plant, *Leptospermum scoparium*, Lyon et al. (1969a,b) found that soluble Cr in leaf tissue was present as the trioxalatochromium(III) ion, $[Cr(C_2O_4)_3]^{3-}$. It is known that even when taken up as Cr(VI), Cr will soon be reduced in the plant to Cr(V), Cr(IV) and finally Cr(III) (Micera and Dessi, 1988). Chromium(III) should be insoluble at the physiological pH, so organic acids must increase the solubility of Cr(III) within the plant, thus enhancing Cr translocation. James and Bartlett (1983a) determined that Cr(III) citrate remains soluble up to pH 7.5. These data suggest that hyperaccumulator plants which translocate more Cr than non-hyperaccumulator plants probably have a different metabolism from that of non-hyperaccumulator plants. More organic complexing agents are produced to increase Cr translocation from root to shoot.

Iron is translocated in plants predominantly as the Fe(III)-citrate complex (Tiffin, 1966), and Ni is readily complexed by organic acids and is very mobile in the plant. The majority of Ni in the plant is present in the leaves rather than in the roots (Baker and Brooks, 1989). Soluble Cr concentration is positively correlated with Fe (Cary et al., 1977a) and Ni (Shewry and Peterson, 1976) in plant leaves and roots. The positive correlation indicates that some Fe or Ni hyperaccumulator plants may also be Cr hyperaccumulators, and implies that Cr may have similar translocation and compartmentation mechanisms to those of Fe and Ni.

There is little information about Cr compartmentation in plants. The vacuole is considered to be the major storage site for most heavy metals (e.g., Cd, Zn, Mn, and Ni) (Wagner et al., 1995). We have limited knowledge about the form and fate of Cr in plants and how plants detoxify Cr; it is important to determine where Cr is compartmentalized. After finding that much of the soluble Cr in leaf tissue of *Lyptospermum scoparium* was present in a complexed form with organic acids, Lyon et al. (1969a,b) assumed the function of the Cr-organic acid complex was to reduce the cytoplasmic toxicity of Cr. There is no conclusive evidence for this assumption.

Mechanisms of Chromium Hyperaccumulation by Plants

Few Cr hyperaccumulator species have been identified to date, which is in contrast with Ni, for which numerous hyperaccumulators have been identified (Baker and Brooks, 1989). The species found to accumulate Cr are largely exotic. Research into the mechanisms of Cr hyperaccumulation is surprisingly scarce. For plants to be able to hyperaccumulate Cr from soil, where most Cr exists as insoluble Cr(III), the plants have to be efficient in a series of processes including solubilization of Cr in soil, absorption of soluble Cr, and translocation, compartmentation, and detoxification of absorbed Cr within plant. The failure of any of these processes will prevent the plant from hyperaccumulating Cr from soils. Given the chemistry of

Cr in the soil, solubilization of Cr could be a limiting process. Several studies have reported that plant uptake of Cr increased with increased soluble Cr in the media (Cary et al., 1977a; McGrath, 1982). If the plant releases a Cr chelator or decreases the rhizosphere pH, both of which both could increase the solubility of Cr(III) in the soil, the plant could have the potential to hyperaccumulate Cr.

The plant species *Leptospermum scoparium* 'J.R. et G. Forst' (Myrtaceae) is an accumulator of Cr and there is a highly significant correlation between plant and soil Cr concentration (Lyon *et al.*, 1969a,b). This species can accumulate up to 20,000 μg Cr g^{-1} in the foliage ash when grown on serpentine soils. Despite the low solubility of Cr, there are other species that contain large amounts Cr (Table 2). Peterson and Girling (1981) reported 48,000 and 30,000 μg Cr g^{-1} in the ash of *Sutera fodina* and *Dicoma niccolofera*, respectively.

Proposed Gene Regulation by Chromium

In preliminary experiments performed at Texas A&M University, dicots have generally exhibited significantly greater Cr absorption rates compared to monocots. Also, dicot species differ significantly in their tolerance to high CrO_4^{2-} concentrations. Some plant species (e.g., sunflower, soybean and clover), treated hydroponically with Cr, exhibited a strong purple color in the stems and leaves, which is due to anthocyanin pigment accumulation in the cellular vacuoles. Thus, it appears that Cr, similar to Cd, induces the expression of *GmGST26-A* in soybean, and this may result in anthocyanin accumulation as well as Cr accumulation. The mechanism of induction is thought to be mediated by a common signal transduction pathway induced by oxidative stress.

TABLE 2

Preliminary List of Plant Species to be Collected for Metal Hyperaccumulation Studies

Nickel Hyperaccumulators

Brassica juncea L. Indian Mustard.

Streptanthus polygaloides Gray (Brassicaceae).

Silene spp.(S.*vulgaris, S. burchelli, S.cobaltica, S.nflata, S. diocia*). (Caryophyllaceae) (>10,000 ppm Ni). Select varieties with the highest dry matter production

Thlaspi s pp. (*T.caerulescens,T. montanum,T. ochroleucum*) (Brassicaceae)

Alyssum spp. (BRASICACEAE) (*A.argentum, A. corsicum, A. euboeum, A. helderechii, A. murale, A. enium, A. troodii*)

Chromium Hyperaccumulators

Leptospermum scoparium J.R &G. Frost. (Myrtaceae) (20,000 ash weight) (2,470 ppm) (small bush, common in New Zealand) (Lyon *et al.*, 1967)

Berkheya coddii (238 ppm dry weight) (Morrey and Balkwill, 1989)

Sutera sp. (Scophulariaceae) aff. *S. silenoides* (Morrey and Balkwill, 1989); Wild,1974)

Sporbolus pectinatus Hack. (Graminae) (Morrey and Balkwill, 1989)

Pimelea suteri Kirk (small endemic plants confined entirely to serpentine area)

Sutera fodina (Fabaceae) (48,000 ppm Cr in ash weight), (Lyon *et al.*, 1967; Wild, 1974)

Andropogn gayanus (Poaceae) (690 ppm Cr in ash weight) (Wild, 1974)

Dicoma niccolifera (30,000 ppm Cr in ash weight) (Lyon *et al.*, 1967)

Cassinia vauvilliersii (Homb et JacQ.) Hook. f.var serpentina (small bush, common in New Zealand) (Lyon *et al.*, 1967)

Hebe odora (Hook. f.) (small bush, common in New Zealand) (Lyon *et al.*, 1967)

(TABLE 2, Continued)

Agronomic Plants

Lolium perenne L., Perennial Ryegrass

Helianthus annuus, Sunflower

Pea mutant (E107)

Sporobolus airoides, Alkali sacaton,

Forage Brassicas

Turnip (*B. rapa* L.), Rape (*B. napus* L.), Swede (*B. napus* L.), Tyfon [*B. rapa* L. x *B. pekinensis* (Lour.) Rupr.].

Nicotiana tabacum, Tobacco

Common weeds

Hemp dogbane, and Ragweed

URANIUM

Natural uranium (U) exists as three isotopes, ^{234}U, ^{235}U, and ^{238}U, with a relative abundance of 0.0055, 0.720, and 99.27 percent, respectively (Allard et al., 1984). In many instances, the natural abundance has been altered either due to anthropogenic U enrichment or geological processes. Uranium is primarily an alpha (a) emitter. Due to the relatively high mass of the a particle versus beta (b) or gamma (g) particles and its lower velocity, the primary mode of exposure is through direct contact with the a emitter. The dangers associated with exposure are mainly through ingestion by inhalation or drinking contaminated water. Uranium behavior is similar to that of other heavy metals, and its physiological toxicities, other than damage from ionizing radiation, mimic that of Pb. Uranium is chemically toxic to kidneys, and insoluble U compounds are carcinogenic (Department of Energy, 1988). Though U and its daughter elements have not been shown to be essential or beneficial to either plants or animals, many plant species will absorb U and incorporate it into their biomass along with other heavy metals (Mortvedt, 1994; Sims and Kline, 1991; Sheppard et al., 1989; Singh and Narwal, 1984; Narwal et al., 1983; Moffet and Tellier, 1977). This observation suggests the possibility for remediation of U-contaminated soils through plant uptake.

Several contaminated sites have drawn considerable national attention. The Rocky Flats plant, near Golden, CO, has soils that are contaminated with U due to improper waste-storage practices (Liator, 1995). Near surface disposal of spent fuel and other low level radioactive wastes from nuclear power-generating plants is accomplished by solidifying the liquid wastes (Jones and Serne, 1995). Leachates from these systems may present problems. The mining and milling of U results in large quantities of waste material called tailings. These tailings consist of overburden from strip and open pit mines as well as the byproduct from ore processed in milling facilities. These materials still contain sufficient amounts of radionuclides to warrant concern for environmental health (Johnson et al., 1980; Sheppard and Thibault, 1984; Tsivoglou and O'Connell, 1964).

The removal of U and other radioisotopes from soils is theoretically simple to achieve. Currently, soil is moved offsite for leaching/chelation treatments, and then returned to its previous location (Entry et al., 1996). However, in practice, the movement of large quantities of soil for decontamination is environmentally destructive and costly due to transportation. Transportation also increases the risk of releasing potentially harmful radionuclides into the atmosphere as particulate matter. Some industries have opted simply to pile the contaminated soil for later remedial treatment (Truan et al., 1989). *In situ* techniques for decontamination of soils are more desirable. Phytoremediation, the removal of contaminants from a soil by actively growing plants, is a potentially feasible alternative to methods currently employed for decontamination. By this procedure, the contaminants are removed from the soil and sequestered within plant tissue. The above ground portion of the plant is then removed and disposed of by incineration, thus reducing the volume of contaminated matter and greatly reducing both the cost and environmental impact of disposal.

An improved understanding of soil/plant interactions and processes involving the solubilization, absorption, transport and accumulation of U contaminants by plants in affected soils is essential for the development of better phytoremedial techniques. Increased uptake of contaminants by plants should lead to more efficient amelioration of afflicted soils. There is an increasing emphasis on the uptake of heavy metals by selected plant species, but few studies have dealt specifically with U.

Uranium Behavior in Soils

Uranium (U) is most mobile as U(VI) (Campbell and Biddle, 1977). It exists in solution predominantly as UO_2^{2+} and as soluble carbonate complexes, $(UO_2)_2CO_3(OH)_3^-$, $UO_2CO_3^\circ$, $UO_2(CO_3)_2^{2-}$, $UO_2(CO_3)_3^{4-}$, and possibly $(UO_2)_3(CO_3)_6^{6-}$ (Ciavatta et al., 1981; Duff and Amrhein, 1996; Grenthe et al., 1992; Langmuir, 1978). Between pH 4.0 and 7.5, the pH range of most soils, U(VI) exists primarily in hydrolyzed forms (Meinrath et al., 1996). Bunzl et al. (1995) found that a significant portion of U contaminant in soils exposed to vented air from U ore mine shafts was incorporated into the clay lattice of the soils. The least amount of U was found in the exchangeable and soluble forms, thereby limiting the amount available for plant uptake and subsequent phytoremediation. In another study, it was observed that in soils spiked with U(VI) as $UO_2(NO_3)_2{\approx}6H_2O$ and analyzed shortly afterwards, the dominating proportion of U(VI) was found in association with the Fe-Mn oxide portion of the soil (Sheppard and Thibault, 1992). The discrepancies between these two studies are probably due to the time-dependent geochemical reactions of U(VI), soil properties, and environmental conditions. Sorption of U(VI) onto soil surfaces tends to increase with increasing pH (up to pH 7) and is readily reversible by decreasing the pH. Uranium(VI) has been shown to be rapidly desorbed from some soils and is competitive for exchange sites with Mn and possibly other divalent cations (Willet and Bond, 1995). A better understanding of the mechanism(s) of reaction of U(VI) in soils will enable better decisions to be made from a remedial standpoint.

Several studies have been conducted on the relationships between plants and soils relevant to radionuclide accumulation by plants (Mortvedt, 1994; Sheppard et al., 1989; Moffett and Tellier, 1977). Plants remove nutrients from the readily exchangeable and soluble fractions of the soil. According to previous work, negligible amounts of U(VI) remain in the soluble and exchangeable forms over any significant period of time (Bunzl et al., 1995; Sheppard and Thibault, 1992). However, one study found that the rate of desorption of U(VI) did increase with time (Sheppard and Thibault, 1992). In this study, it was also observed that the insoluble organic matter fraction of the soil formed strong complexes with U(VI).

Uranium Behavior in Plants

Little information is available on the accumulation of U in plants except in the literature related to the use of native plant species in the biological exploration of metals. However, there are studies related to the mechanisms by which plants absorb and accumulate U (Boileau et al., 1985; Campbell and Rechel, 1979; Saric et al., 1995; Sheard, 1986a,b; Sheppard et al., 1984; Titaeva et al., 1979; Zafrir et al., 1992). In these studies, it was generally observed that plant species differ in U accumulation. Uranium accumulates mainly in the roots and depth of U placement and soil properties influence absorption by plants. There is contradictory information on the phytotoxicity of soil U to plants (Sheppard et al., 1992). Levels as low as 1 mg kg^{-1} in soil, well within the normal background range, have been cited as toxic. Gulati et al. (1980) reported that the highest yield of wheat was obtained at 3.0 mg U kg^{-1} and that tomato yield decreased continuously with increasing U level in soil from 1 to 6 mg kg^{-1}. Other studies have reported no toxicity at levels 100- to 1000-fold higher. For example, *Brassica rapa* produced seed and high biomass yields at U levels of 10,000 mg kg^{-1} (Sheppard et al., 1992) in soil. To identify the toxic threshold of soil U, Sheppard et al. (1992) tested nine levels of U in 11 soils with 5 plant species. They found no detrimental effects below 300 mg U kg^{-1} in soil.

Recently, there have been reports of the high bioavailability of U to agricultural plants. Sunflower was shown to be very effective in recovering U from U-contaminated water. Uranium accumulated mainly in the roots, with concentrations 5000 to 10000 times greater than that in the water (Entry et al., 1996). Huang et al. (1997a) reported that they have developed remediation technologies for the clean up of U-contaminated soils and techniques to trigger U hyperaccumulation in plants. They observed that U accumulation in plant shoots increased by more than 1000 fold by use of organic acids. Shoot U concentration of *Brassica juncea* increased from 5 mg kg^{-1} to more than 5000 mg kg^{-1} with organic treated soil.

PLUTONIUM

Plutonium (Pu) has been used predominantly in weapons manufacture. It can be found in minute quantities globally as it is a fallout material from nuclear weapons testing. Previous work on Pu has focused primarily on migration and transport mechanisms, with little emphasis on the mechanisms of root uptake and plant accumulation. Primary interest has been in determining mechanisms by which food crops could have become contaminated and the risks of bioaccumulation, with little interest in determining species of plants that might be suitable for phytoremediation. The danger inherent when working with above background quantities of Pu has prevented scientists from studying it very closely and determining the mechanisms of plant uptake and the factors affecting its availability to plants. The soil chemistry of Pu is not as well known as that of most other metals. Strict regulations for the procurement of special nuclear materials (SNM) have severely limited the amount of Pu available for study.

Behavior of Plutonium in the Soil/Plant System

Downward leaching of Pu in the soil profile is relatively slow (Bunzel et al., 1992; Muller, 1978). Chelation by naturally occurring soil organic constituents was proposed as the most likely mechanism of mobility of Pu and subsequent uptake by plants (Bondietti et al., 1976; Francis, 1973). Assumptions were made that Pu associated with low molecular weight molecules was more mobile and thus readily available for plant uptake. Plutonium associated with higher molecular weight molecules was assumed to be non-mobile and not plant available (Livens et al., 1987). Further research showed that the amount of Pu assimilated by tumbleweed

(*Sasola* sp.) increased in Pu contaminated soils treated with DTPA, with up to a 1000-fold increases in uptake reported (Ballou et al., 1978). Vyas and Mistry (1983b) found significant increases in plant uptake in both an oxisol and a vertisol when Pu was supplied as Pu-DTPA compared to Pu-EDTA or Pu nitrate. Vyas and Mistry (1983a,b) also found a relative decrease in the quantities of Pu-DTPA, Pu-EDTA, and Pu nitrate assimilated by plants grown in nutrient solution compared to soil. This phenomenon could be an indication of competitive absorption by another cation that was limited in the soil, but not in the nutrient solution. In long term field studies, ^{238}Pu and ^{239,240}Pu concentrations in a soil remained constant with depth over years, an indication of little vertical leaching through the soil profile. Plutonium probably undergoes reactions within the soil shortly after contamination, which would make it less available for absorption by the plant. Plutonium is more mobile in coarser textured soils and less so in peats and mucks (Federov et al., 1986; Mahara and Matsuzuru, 1989). Wind transportation and migration have been identified as the major causes of Pu movement in agricultural ecosystems as well as the major source of shoot contamination (Federov et al., 1986; Little et al., 1980; Little and Whicker, 1977; McLeod et al., 1980; Pinder et al., 1990).

Relatively little information is available that pertains to the soil reactions of Pu. It has been shown that the solubility and extractability of Pu(IV) is affected by carbonate concentration in solution (Nishita and Hamilton, 1980). In systems containing no carbonate, an increase in the quantity of extractable Pu corresponded to an increase of pH to 8-10. The explanation offered was that the dissolution of alkali soluble portions of soil organic matter, and possibly the dissolution of hydrous sulfide which existed in association with Pu, were responsible for the increased amount of Pu extracted. The percent of Pu recovered from the organic matter fraction was reduced from 94 percent at pH 7.1-12.3 in systems containing no carbonate to 0.1 to 1.3

percent recovery in systems where carbonate was present. Free $CaCO_3$ in all treatments drastically reduced the extractability of Pu, indicating possible associations with calcite. Under acidic ($pH < 3.0$) or alkaline ($pH > 7.0$) conditions and in the presence of a high percent of organic matter, Pu becomes more mobile in a kaolinitic soil. This phenomenon is likely due to the dissolution of acid and alkali soluble organic matter. Above pH 6.0, little Pu was extracted from kaolinitic soils with small amounts of organic matter, but extractable Pu increased with decreasing pH (Nishita et al., 1978). Little (approximately 3 percent) of the Pu present in the soil was readily exchangeable. The amount of Pu in association with the organic fraction of soil was variable (2 to 13 percent, depending on pH) with the majority (73-88 percent) associated with the reductant soluble fraction (Fe-Mn oxides) and the remainder (approximately 19 percent) residing within the mineral lattice (Muller, 1978). The chemistry of Pu in the soil system is influenced by pH, organic matter content, and mineralogy, including aluminosilicate, free iron oxide, free silica, and alumina contents.

Plutonium Uptake by Plants. In general, the information available on this topic has been from a dosimetry standpoint, with little emphasis on developing an understanding of the mechanism(s) involved in the uptake of Pu by plants. Agricultural plants were investigated for their ability to accumulate Pu and the potential migration of Pu into humans due to ingestion of agricultural products. More Pu was found in the seeds of soybean (glycine max) (Adriano et al., 1982) than would normally be expected. This was likely due to harvesting practices, in which the suspension of airborne particles and subsequent deposition onto the seed contributed to the quantity of Pu present.

Pimpl and Schuttelkopf (1981) performed a literature search to evaluate the magnitude of transfer factors (concentration ratio) for Pu. They found transfer factors in the range of 10^{-9} to

10^{-3}. The transfer factor was dependent on the cation exchange capacity of the soil and the pH. Complexing agents increased the transfer factors by up to 1300-fold. Transfer factors or concentration ratios (CR) were found to be higher than expected in the shoots of rice grown in non-amended soils, but results similar to the expectations of the investigators were obtained for the grain (Adriano et al., 1981). Wildung and Garland (1974) reported that the concentration of Pu in roots exceeded that in the shoots by factors of 3 to 8, depending on soil Pu concentration, and Pu was shown to be translocated downward in the roots after uptake from the soil. Lipton and Goldin (1976) reported that increases in plant uptake of Pu due to chelation were of the order of 1000 fold. Regardless of the form of ^{238}Pu contaminant used, it has been shown that the assimilation rate by the plant is constant (Brown and McFarlane, 1978). In winter wheat (*Triticum aestivum*), it has been established that up to 70 percent of the contamination of grain by Pu is due directly to the redeposition of dust during harvesting (McLeod et al., 1980). McLeod et al. (1981) found that in different cropping rotations (winter wheat - soybean, fallow - corn, continuous bahai grass, and continuous white clover) grown on the same contaminated soils, that liming decreased the assimilation of Pu by all crops at all soil concentrations of Pu. It was also determined that the assimilation rate by the plant was independent of the concentration of Pu within the soil (McLeod et al., 1981). Further research is essential for a better understanding of the mechanism(s) of plant uptake of Pu to aid in the process of phytoaccumulation as a remedial action.

DEPARTMENT OF ENERGY INVOLVEMENT IN DEVELOPING PHYTOREMEDIAL TECHNOLOGIES

Research funded and/or conducted by DOE has paved the way for present day remedial techniques using phytoextractive techniques. Ongoing projects are multidisciplinary approaches to phytoremediation.

One such project involves the Amarillo National Resource Center for Plutonium in Amarillo, Texas (UTA96-0043). Investigators L. R. Hossner, R. H. Loeppert, and R. J. Newton (Texas A&M University) and P. J. Szaniszlo (The University of Texas) are working in collaboration with Moses Attrep, Jr., of Los Alamos National Laboratory, to: (a) screen native and agricultural plant species for their ability to accumulate Cr, Pu, and U, and (b) identify plant biochemical and physiological characteristics associated with the tolerance to and accumulation of the target metals. Approximately 50 agricultural and native species, including plants previously identified for their ability to hyperaccumulate heavy metals, are being evaluated in soil and hydroponic culture for their tolerance to and ability to absorb, accumulate and translocate the target metals. Selected plant species are evaluated with respect to: (a) kinetics of absorption of inorganic and organically complexed forms of the target metals, (b) localization of absorbed metal in the plant, (c) influence of the well characterized P- and Fe-deficiency stress responses on target metal absorption and translocation, and (d) the predominant mode of metal binding within the plant. Evaluation of the potential of agricultural and native plants to accumulate radionuclides and their analogs is important for the containment of any released pollutants, the prevention of such from endangering natural ecosystems and nearby human populations, and the development of phytoremedial strategies.

The Argonne National Laboratory is currently involved in work to further the efficiency of phytoremediation (P/ANL—000703). Investigation of bioremediation of contaminated soils by enhanced plant accumulation is underway. Modified agricultural techniques and multiple cropping systems to enhance the ability of the plants to accumulate target contaminants are being developed. Woody and herbaceous plants will be selected under greenhouse conditions and laboratory studies conducted for the ability of plants to accumulate target contaminants. Soil amendments, such as chelates, will be evaluated for their ability to enhance mobility and plant accumulation. Accumulation factors in various soils will be evaluated along with the optimal nutritional needs of the accumulating plants in different soils. Argonne National Laboratory is working in collaboration with Applied Natural Sciences, Inc., of Hamilton, Ohio.

Dr. David W. Ow of the Plant Gene Expression Center, Agricultural Research Service (USDA), is investigating the molecular genetics of metal detoxification and their prospects for phytoremediation. Dr. Ow will identify target genes in a model organism (*Schizosaccharomyces pombe*). Modification of these genes for expression in a heterologous host will be used to enhance the hosts ability to accumulate metals. Identification of these genes will also aid in the search for more complex organisms exhibiting these same genes. Also, the elucidation of metal sequestration and detoxification within the fungi will assist in the engineering of fungi to aid in phytoextraction.

Dr. Julian I. Schroeder, of the Department of Biology at the University of California at San Diego, is molecularly characterizing a novel heavy metal uptake transporter from higher plants and evaluating its potential for use in phytoremediation. Dr. Schroeder will investigate the role of cDNA (named PMT1) in a plasma membrane heavy metal and calcium uptake transporter isolated from plant roots and the possibility that overexpression of PMT1 cDNA in plants can be used to enhance plasma membrane heavy metal uptake into plant tissues. Through isolation of PMT1 cDNA, Dr. Schroeder will address questions regarding mechanisms of heavy metal uptake across plasma membrane of plant cells. Dr. Schroeder will test his hypothesis by: (a) characterizing the selectivity of the cDNA for uptake of toxic heavy metals, (b) determining if PMT1 itself represents a metal transporting membrane protein, (c) the use of low stringency hybridizations to determine if PMT1 is a member of a larger gene family, and (d) overexpression of PMT1 and PMT1 mutants that enhance heavy metal uptake to determine whether PMT1 expression can enhance heavy metal uptake.

Dr. Teresa W. M. Fan, of the Department of Land, Air and Water Resources at the University of California at Davis, will investigate the plant rhizosphere effects on metal mobilization and transport. The objectives of Dr. Fan's research include: (a) determination of the biochemical composition of root exudates with a particular focus on metal-binding organic acids and the changes in their composition and biosynthesis in response to selected metals, (b) application of the analytical methodology gained in the previous objective to investigate the influence of mycorrhizae on root exudation and metal uptake from contaminated soil in soil microcosms. Dr. Fan proposes that once the root exudates or cell wall components that are involved in metal mobilization/transport are identified, it will be feasible to examine the metabolic control of their synthesis to determine how microbial activities influence the process. Dr. Fan also believes that this will provide a rapid method for screening plants for increased metal mobilization and tolerance and possibly an examination of enhancement of metal mobilization through breeding and other processes used to theoretically enhance a plants phytoremedial capacity.

Phytotech Inc., of Monmouth Junction, NJ, has been developing phytoremediation for a number of years as a practical, effective process for the remediation of soils contaminated with target metals. Phytotech, Inc. has several species of plants ready for commercial decontamination of Pb-contaminated soils. Soil amendments, presumably EDTA application, and manipulation of soil pH have led the company to what is hoped to be an efficient method for decontaminating soils. Currently, DOE funded projects include mobilization and removal of cesium (Cs) and strontium (Sr) from soil by chemical treatment and phytoremediation (Project ID: P/CH- - FG02 - 96ER86401). Phytotech Inc. will improve methods of Cs and Sr solubilization by the addition of soil amendments prior to their removal by plants. Screening for hyperaccumulator plants will also occur under greenhouse conditions to describe the potential of different plant species to accumulate Cs or Sr.

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