CHAPTER 2

LITERATURE REVIEW

This chapter comprises several factors affecting the Cd uptake of plant, uptake mechanism, physiological responses and amelioration of contaminated soil using plants. It should be noted that though this study absolutely involved the effects of Cd on plants grown hydroponically in controlled conditions, it is necessary to know the factors such as soil properties and other heavy metals effects. This is because in the field conditions terrestrial plants take up Cd from soil and the uptake is controlled by these relating factors.

2.1 Factors affecting plant uptake of cadmium

One of the major pathways by which Cd enters the food chain is via plant uptake of this metal from soil. The risk of exposure is not easy to assess simply from analysis of metal concentrations in soil, since availability will depend on many factors which relate to soil, microbial activity and plant itself. These factors are as follows.

2.1.1 pH : Soil pH is the major factors determining the availability of Cd in the soil because it affects all adsorption mechanisms and the speciation of metals in the soil solution (Alloway, 1990). Soil pH was inversely related to Cd accumulation in wheat grain. A negative linear relationship for soil pH and grain Cd existed between soil pH 5.0 to 6.2. At soil pH < 5.0 grain Cd remained constant at 45 μg/kg, while at soil pH > 6.2 grain Cd remained constant at 9.9 μg/kg. These findings reveal the increase in Cd solubility and bioavailability under acidic conditions (Gavi, Basta and Raun, 1997). In contrast, the solution culture experiment has shown that pH differences (pH 4-7) has only a small effect on the Cd uptake of corn with some evidence of inhibition of absorption and translocation at low pH. The H^+ does not compete effectively with the Cd^{2+} for uptake (Tyler and Mcbride, 1982).

2.1.2 Cation Exchange Capacity (CEC) : The Cd content of plant is inversely proportional to the CEC of the soil (Haghiri, 1974). However, Alloway (1990) reported that the relationship between CEC and plant uptake remains unclear because cation exchange is only one of several adsorption mechanisms affecting the solubility of Cd in soils.

2.1.3 Cadmium origin and its content : Alloway (1990) reported that crops grown on soils spiked with Cd salts take up more Cd than those grown on soils containing the equivalent amount of Cd from sewage sludge. Uptake by maize was 5 to 18 times greater from $CdSO_4$-spiked soils compared with equivalent amounts of Cd in sludge. In addition, increasing amount of this metal is more likely to affect the Cd content of plant although the availability of Cd depends on many factors. Logan and Feltz (1985) supported the hypothesis that at a given Cd application rate Cd uptake should decrease at Cd content of the sludge decreases. Jing and Logan (1992) suggested that the quality of sludge might be as important as soil pH and Cd loading rates in regulating food chain contamination from Cd in sewage sludge. The 'clean' sludges, those low in Cd (and presumably other contaminants) pose less of a risk to the food chain than more contaminated sludges at equal sludge application rate.

2.1.4 Organic matter : The presence of humic acid in solution cultures containing Cd decreased the Cd activity in solution and marginally suppressed its absorption by corn roots (Tyler and Mcbride , 1982). As for the role of organic matter in soil, Haghiri (1974) pointed out that although organic matter has a high CEC and has the capacity of adsorbing large quantities of Cd, its effect is not permanent. For example, by annual land application of large quantities of material such as sewage sludge which is very high in organic matter and contains Cd (depending on the source), one should not expect any crop damage from Cd. However, over a number of years the net concentration of Cd in the soil will increase even though Cd is not available to plants. When the addition of organic matter is ceased, the organic matter content of the soil will decline. The concentration of Cd in soil solution will probably continually increase and eventually may exceed the threshold for crop production. This is consistent with the 'sludge time bomb hypothesis' which postulates that metal adsorption capacity of the soil is augmented by soil organic matter added as sewage sludge, but this capacity will revert back to its original background level with time following termination of sewage sludge application as slow mineralization of organic matter in sludge releases metals in more soluble forms. This hypothesis together with the plateau theory has been proposed to describe the phytoavailability of potentially toxic metals in sewage sludge treated soil. Both of them were discussed by Chang, Hyun and Page (1997). The plateau theory argues that the metal adsorption capacity added with sludge will persist as long as the metals of concern persist in the soil and the metals would remain in chemical forms not readily available for plant uptake. Therefore, the metal concentrations of plant tissue will reach a plateau as sewage sludge mass loading increases and remain at this plateau after termination of sludge application. On the other hand, the sludge time bomb hypothesis does not dispute the possible presence of a plant tissue metal concentration plateau during the course of applying the sewage sludge on cropland. It addresses the possible consequences for metal concentration in plant tissue following the termination of sewage sludge application. Since soils have a finite capacity to immobilize metals by adsorption or precipitation reactions without the protective effect of the sorptive material in the sludge itself, the Cd concentration in plant tissue will then rise rapidly. But from 10 years of experimental data, the results indicate that an actual plateau or time bomb was not evident but condition for plateau or time bomb to take place may be found.

2.1.5 Other ions : The effect of ions on the Cd uptake is less clear. Copper, Ni, Se, Mn and P have an antagonistic effect on Cd uptake. Nevertheless, Pb is considered to have a synergistic effect due to it being preferentially adsorbed. Zinc has been found to have an antagonistic effect on Cd uptake in soils with low Cd concentration, and either a synergistic or a nil effect with relatively high Cd contents (Alloway, 1990). The effect of Zn is against the earlier work conducted by Haghiri (1974). Jing and Logan (1992) found no significant correlations of Cd uptake with Cd/Fe, Cd/Al or Cd/(Fe+Al) ratios. There was, however, a higher correlation of plant uptake with Cd/P ratio. While Gabi, Basta and Raun (1997) reported that long term use of N and P fertilizer did not raise Cd level of wheat grain.

2.1.6 <u>Mycorrhiza</u> : There are some reports about the reduction of Cu, Ni and Zn toxicity by mycorrhiza. Root infection by mycorrhiza enhances metal tolerance. Amelioration of toxicity appears to involve a reduction in the amount of metal available to the plant by incorporation into the fungus, thereby reducing uptake. The roots of many plant species which may colonize metal-contaminated soil can be infected with mycorrhiza (Davies , 1994). Although there is no discussion about the fungus role on reducing Cd toxicity from Davies' review, it is possible to obtain the similar result.

2.1.7 <u>Plant factors</u> :

a) Plant genotype : Plant species and varieties differ widely in their ability to absorb, accumulate and tolerate heavy metals. Lettuce, spinach, celery and cabbage tends to accumulate relatively high concentrations of Cd, whereas potato tubers, maize, french beans and peas accumulate only small amounts of Cd (Alloway, 1990).

b) Root exudates : The release of organic exudates by the plant root may influence nutrient solubility and uptake indirectly through their effects on microbial activity, rhizosphere physical properties, and root growth patterns, and directly by acidification, chelation, precipitation and oxidation-reduction reactions (Uren and Reisenauer, 1988 cited in Krishnamurti et al., 1997). Excretion products of roots include a variety of low-molecular-weight organic acids such as citric, oxalic, tartaric and acetic acids. These exudates may contribute to the formation of soluble complex and chelates and modify the mobility of the heavy metals in the soil rhizosphere. Chelation of metals in soil disturbs the equilibrium between the labile metal on the solid phase and soil solution and thus enhances the release of the former to the soil solution. Therefore, metal chelates in the soil-root interface zone replenish the metal ion taken up by the plant. Removal of a chelated metal by plant uptake thus establishes a diffusion gradient to transport more chelated metal toward the root surface. The use of 0.01 M of these organic acids increased the Cd release up to a reaction period of about two hours and then slowly decreased with time by microbial degradation. The Cd release was in the order : furmaric acid >citric acid > oxalic acid > acetic acid ≅succinic acid. The kinetics of Cd release from the soils was diffusion controlled, and the dynamic release of these low-molecular-weight organic acids from the plant roots into the soil rhizosphere would continuously release Cd from the soils (Krishnamurti et al., 1997).

2.2 Cadmium uptake and translocation

Uptake of cationic solutes is likely to driven largely by the negative membrane potential across the plasma membrane, which is generated in part by metabolically dependent processes such as proton extrusion via the plasma membrane H^+ATPase. Carrier-mediated uptake has been reported for a number of cationic micronutrients. Cadmium is not known to be an essential plant micronutrient, it is noteworthy that Cd uptake appears to occur via a carrier-mediated system. It is evident that Zn appears as a strong competitor of Cd in root uptake; therefore, it shares common transport sites or processes (Cataldo, Garland and Wildung, 1983; Costa and Morel, 1994; Hart et al., 1998). Cataldo et al. (1983) investigated absorption characteristics of Cd^{2+} in soybean . It was found that at concentration of 0.0025 to 0.5 μM the fraction of non-exchangeable Cd bound to roots was 20 to 25% of the absorbed fraction, and increased to 45% at solution concentration in excess of 0.5 μM . While the metabolically absorbed fraction was shown to represent 75 to 80% and 55% at concentration less than 0.5 μM and at 5 μM, respectively. These results were confirmed by Costa and Morel (1994). Cd uptake by four cultivars of lettuce revealed that uptake processes depending on ATP seemed to be important and was especially true at low Cd concentrations in the medium. At concentrations higher than 0.1 μM Cd the contribution of active processes involved H^+ATPase was reduced, suggesting that Cd entered the cell by other processes such as an irreversible binding of Cd to root exchange sites or cell wall material. However, the exchangeable fraction due to the presence of anionic charges, i.e. carboxylic groups, located in the apoplasm of the root, was lower than that found with soybean seedlings. Translocation of Cd to shoots in durum wheat cultivar compared with the bread wheat cultivar was investigated by Hart et al. (1998). It was found that lower shoot Cd accumulation was observed in durum wheat cultivar though Cd content in roots was higher. This indicates that Cd is retained in the roots, perhaps by a mechanism involving sequestration or decreased xylem loading of Cd. Cadmium is known to accumulate in the vacuoles of root cells via more than one mechanism. Movement of Cd across the tonoplast of oat root cells has been described as occurring by a Cd^{2+}/H^+-antiport system as well as by a phytochelatin-Cd transporter that may be Mg-ATP dependent. Whatever the mechanism of tonoplast Cd transport, vacuolar compartmentation of Cd would tend to limit symplastic movement of the heavy metal. Although excess Cd accumulation in durum wheat grain was not correlated with seedling-root influx rates or root-to-shoot translocation, the similar mechanism was manifested in the durum grain in which Cd that has been loaded was less likely to be remobilized out of grains. This would imply that symplastic transport processes are of primary importance in understanding Cd accumulation in wheat grains. Another process involved in Cd movement into wheat grains is likely that loading into developing grains occurs via the phloem.

2.3 Effects of cadmium stress on physiological responses

There are three different stages broadly involved in the uptake and movement of Cd solution in plants. The first short-term stage occurs within a few hours of exposure, the primary effect being on root metabolism and growth; an indirect effect is the stomatal opening generated by the increase of the osmotic potential of the leaves. The second stage occurs 1 to 2 days after exposure to Cd. Cadmium acts directly on the guard cells, and severe root growth inhibition limits water uptake and causes a decrease in relative water content of the leaves and a stomatal closure. When exposure continues for a long time or very high Cd concentration are applied, there is a general metabolic decline with a loss of turgor and a hydropassive stomatal closure (Barcelo et al., 1986 cited in Marchiol et al., 1996). High level of heavy metals, including Cd, induces an ion stress in plants clearly distinct from salt stress. An excess of these metal ions or of soluble metal chelates may induce a series of biochemical and physiological alterations in plants which present some common characteristics. Membrane damage, alteration of enzyme activities and inhibition of root growth are considered characteristic features of heavy metal stress. These early events lead to a large range of secondary effects, such as disturbance of hormone balance, deficiency of essential nutrients, inhibition of photosynthesis, change in photoassimilate translocation and alteration of water relations, which further enhance the metal-induced growth reduction (Barcelo and Poschenrieder, 1990).

2.3.1 Cell expansion as affected by cadmium

Study on Cd distribution in maize roots has shown that most of Cd is associated with cell walls and the middle lamellae. Increased cross-linking of pectins in the middle lamellae may increase cell adhesion, which would be a significant source of resistance to growth. Decreased cell wall extensibility may also arise from metal-induced decrease of cell wall synthesis.

Formation of polynucleated cell occasionally occurred in Cd treated plants, which suggests that cell wall synthesis is limiting cell growth (Barcelo and Poschenrieder, 1990).

2.3.2 Alteration of plant-water relation

2.3.2.1 Water transport via plasma membrane

Water channels or aquaporins play significant role in transport of water via membrane. This transport is governed by an alteration of water channel protein activities and a change in its content (Chrispeels and Maurel, 1994). Water channel are thought to be selective mainly because they are quite narrow, thus allowing the passage of water molecules one by one in single file. Even polar solute molecules with diameters slightly bigger than those of water are thought to be excluded from entering the pore. In plant tissue water channels and not plasmodesmata may mediate most of the water flow from cell to cell and the transcellular rather than the symplastic component would be more important than the apoplastic. Factors which can trigger the opening and the closing of channel by the change in conformation of transport protein are heavy metals, high concentration, low water potential and the turgor. Besides, the composite structure of membrane with proteinaceous arrays in parallel with lipid arrays suggests that the inhibition of water channels do not affect solute flow (Steudle and Henzler, 1995).

2.3.2.2 Effect of cadmium on factors governing plant water balance

Root growth and morphology

Root growth is the result of two different mechanisms, cell division at the root tip and cell elongation in the extension zone. It is noted that stone pine (*Pinus pinea* L.) and the maritime pine (*Pinus pinaster* Ait.) grown in culture solution supplied with 5 μM $CdSO_4$ had dramatically reduction in tap root elongation. A supply of 0.1 or 1 μM Cd^{2+}, however, enhanced root elongation in stone pine without significantly influencing root elongation in maritime pine. In both species the root density and the width of the cortex increased in response to Cd^{2+} exposure . The thickening of the root suggests that heavy metal toxicity affects cell elongation in extension zone more than cell division in the apical meristem (Arduini, Godbold and Onnis, 1994).

Impaired spatial distribution and the reduced root hair surface of the metal stressed roots lead to bad root-soil contact and lower the capacity of plants to explore the soil for water and nutrients (Barcelo and Poschenrieder , 1990). This is because most of the water is absorbed in the root hair zone which is about 67% of total root surface area. Plant root system under metal stress differs from the one facing drought stress, which is more likely to penetrate to the moist deeper soil (Taiz and Zeiger, 1991). In addition, reduced water uptake by metal stressed plants may be caused by an increased resistance to water flow into and within roots. Root browning due to enhanced suberization or lignification may limit water uptake (Barcelo and Poschenrieder, 1990). This response is observed in water stressed plant (Taiz and Zeiger, 1991).

Diameter and number of vessels

The flow of water in the xylem obeys Hagen Poisseuills's law :

$$\text{Flow rate} = (P_2 - P_1)(\pi r^4/8L\eta)$$

Where η is the viscosity of the sap, P_2-P_1 is the pressure gradient along the capillary, L is the length of the pathway, and r is the radius of the capillary. This means whenever there is a decrease of the tracheary diameter, there would be a considerable decrease in conductivity. Toxic levels of Cd, Zn, Al and Cr have been found to decrease vessel diameter in diverse plant species. In bean plant stems, 44.5 µM Cd decreased both vessel radius and the number of vessels, resulting in decreased total vessel area. Other environmental stresses, such as drought, also induce the formation of small vessel, but this decrease may be compensated by an increase of the number of vessels. The decrease of both number and size of vessels in Cd-treated plants may be caused by the metal induced inhibition of cell division in the procambium and cambium, by the inhibition of cell elongation and by metal-induced alterations of the formation of secondary cell wall thickenings (Barcelo and Poschenrieder, 1990).

Transpiration

Studies with epidermal peels floating on Pb, Cd, Ni or Al containing solutions have shown that heavy metals may induce stomatal closure. Experiments on excised leaves have demonstrated that metals increase stomatal resistance not only when directly applied to guard or epidermal cells but also when reaching the leaves via xylem. But in whole plants the increased stomatal resistance may merely be a consequence of early toxicity effects in roots and stems, leading to decreased water availability in leaves (Barcelo and Poschenrieder, 1990). The results from two cultivars of soybean grown in nutrient solution containing Cd for six days indicated that the stomatal conductance was depressed by 50% and 30%. The stomatal width for the Cd-treated plants was significantly less than that of the control, indicating stomatal closure (Marchiol et al., 1996). At low Cd concentration (0.01-0.1 μM), the stomatal conductance of Cd-treated lettuce increased. However, four times decrease of stomatal conductance from 200 μM Cd treatment compared with the control was observed (Costa and Morel, 1994). In addition, increased stomatal density has been observed in *Phaseolus vulgaris* exposed to toxic Cd concentration, but transpiration was decreased (Barcelo and Poschenrieder, 1990).

2.3.3 Translocation of other nutrients

For plants Cd is one of the most dangerous heavy metals due to its mobility and the small concentrations at which its effects begin to show. Synergistic and antagonistic effects can be produced giving rise to changes in the distribution of nutrients in plants compared with those grown in the absence of Cd. Interactions between Cd and other nutrients are seen as changes in the nutrient content of the plant and manifests by physiological disorders and the diminution of growth and yield (Moral et al., 1994). Symptoms of toxic effect are chlorosis, necrosis, and curled leaves with reddish veins and petiole together with growth reduction (Fergusson, 1990). Pea plants grown in perlite and vermiculite cultured with nutrient solution containing 0-6 mgCd/l had a severe decrease in Mn content in nodules. Probably Cd displaced Mn from its metabolic positions. Still, there was a higher translocation of Mn from root to shoot when Cd concentration in the culture medium increased (Hernandez, Garate and Carpena-Ruiz, 1995). The reduction of Mn content in root and shoot was observed in birch (*Betula Pendula*) grown in nutrient solution with 2 μM $CdCl_2$ for six days. Moreover, root concentration of K and Ca and shoot concentration of Ca, P, Fe and Cu was significantly reduced by Cd treatment. But S content in fine roots was increased. Decrease K concentration may be due to effects on the ATPase, responsible for the proton gradient needed to take up K^+ actively. At low external K^+ concentration the uptake of K^+ is mainly energy dependent, and has been shown to be sensitive to Cd. The uptake of polyvalent cation, e.g. Ca^{2+} and Mg^{2+}, is readily depressed by the presence of other polyvalent cations, and Cd has been shown to reduce the uptake of Ca in sugar beet (*Beta vulgaris*), for example. Cations and, especially, the polyvalent ones that are exported to the shoot, mainly enter the xylem through the young roots with unsuberized endodermal cell walls. This means that the apical part of fine roots may be particularly susceptible to heavy metal exposure, thus imparing cation transport to the shoot. Moreover, increased root concentration of S and Cd after Cd exposure suggests a Cd-induced synthesis of Cd-binding sulphur containing peptides in the root tips (Gussarsson et al., 1996).

2.3.4 Proline accumulation

It is generally known that plant cells which actually experience desiccation to the point of turgor loss must regain turgor through osmotic adjustment to resume growth. In other words, it is a reduction of water potential without decrease in turgor. That is, osmotic potential is changed by the rise in solute content/cell, not resulting from alteration of cell volume. The increased solutes include sugars, organic acids and ions, especially K^+. Most ions, affecting enzyme activities, accumulate in vacuole, whereas other solutes build up in cytoplasm to balance the water potential of the cell without interfering the enzyme activities (Taiz and Zeiger, 1991). During a period of water deficit different organisms accumulate a range of amino acids to a greater or lesser degrees, but the most frequent and extensive response is an increase in the concentration of amino acid, proline (Bates, Waldren and Teare, 1973; Stewart and Larher, 1980; Paleg and Aspinal, 1981; Handa et al. , 1986 ; Naidu, Aspinall and Paleg, 1992).

Paleg and Aspinall (1981) reported that proline may accumulate in all organs of the intact plant during water deficit, although accumulation is most rapid and extensive in the leaves. Once the water deficit is eliminated, a fall in proline concentration is observable within 3 to 4 hours to several days depending on plant species. Physiological advantages derived from the accumulation of proline are discussed as follows.

a) Accumulated proline acts as a compatible solute regulating and reducing water loss from the cell during the episodes of water deficit. Such mechanism can be important in a mesophytic higher plant growing in a field environment where diurnal fluctuations in leaf water potential occur.

b) Free proline does not interfere with plant metabolism at the concentrations accumulated during stress.

c) Accumulation of proline in stress situations has evolved as a means of conserving both energy and nitrogen in a readily available form.

d) Metabolically useful role of proline is as a sink for the nitrogenous compounds derived from the net loss of protein. Protein synthesis is readily inhibited by water stress, but protein hydrolysis is not inhibited and may even be enhanced. Proline can protect enzymes against the deleterious effects of biologically toxic compounds such as urea.

In addition to water stress, proline may accumulate in response to other environmental stress factors including high and low temperature and salinity (Stewart and Lee, 1974; Stewart and Larher, 1980; Paleg and Aspinall, 1981), pathogen infection and mineral deficiency (Stewart and Larher, 1980) and heavy metal (Saradhi, 1991; Kastori, Petrovic and Petrovic, 1992; Costa and Morel, 1994; Bhattacharyya and Choudhuri, 1994; Schat, Sharma and Vooijs, 1997). These studies have shown that Cu is the most effective inducer of proline accumulation in plants, while Cd is the second effective. However, it is controversial whether proline accumulation results from metal-induced water stress or only because of toxic effect of metals.

2.3.5 Chlorophyll content

A number of authors reported the reduction of chlorophyll content by the effect of Cd stress (Boonfahprathan, 1983 ;Stobart et al., 1985; Babu and Singh, 1992; Keshan and Mukherji, 1992; Somashekaraiah, Padmaja and Prasad, 1992; Kalita, Devi and Bhattacharya, 1993; Bhattacharyya and Choudhuri, 1994). Cadmium ion inhibits the biosynthesis of chlorophyll by reacting with essential thiol groups in both the protochlorophyllide reductase protein and the enzymes involved in the light dependent synthesis of 5-aminolaevulinic acid (Stobart et al., 1985). Another study has shown that Cd^{2+} decreases the formation of chlorophyll by interacting with sulfhydryl-requiring enzymes like 5-aminolaevulinic acid dehydratase and porphobilinogen deaminase leading to the accumulation of intermediates of chlorophyll synthesis like 5-aminolae-vulinic acid and porphyrins (Padmaja et al., 1990 cited in Somashekaraiah et al., 1992). The experiments conducted on light-grown seedlings revealed that not only the inhibition of chlorophyll synthesis by reaction with constituent biosynthetic enzymes, but the lipoxygenase-mediated accumulation of lipid peroxides and the inhibition of free radical scavenging enzymes like superoxide dismutase and catalase also caused a pronounced reduction in the chlorophyll levels of mung bean (*Phaseolus vulgaris*) seedlings (Somashekaraiah et al., 1992).

In contrast, recent study reported the increase in chlorophyll level with increase in treatment concentrations of both Hg and Cd in *Phaseolus aureus* Roxb. at the germination stage. Notwithstanding, at the seedling stage the chlorophyll content exhibited only an insignificant increase in response to increasing metals concentrations. The increase might be due either to an increase in the number of chloroplasts per cell or a decrease in the cells' volume leading to rising of the numbers of cells per unit weight, or both (Shaw, 1995).

2.3.6 Cadmium accumulation

Cadmium accumulation in plant tissue depends on many factors as described in 2.1. Plant grown by hydroponic technique are capable of accumulating higher level of metal. Of all the factors, however, plant species might be predominant. It is evident that each species varies in Cd tolerance. The more tolerant ones have more tendency to accumulate the toxic metal though some plants can tolerate by excluding it. The accumulations of Cd are summarized in Table 2.1.

Table 2.1 Cadmium accumulation by different cultivated plants

Species	Cd accumulation (ppm)	Cd concentration in medium (ppm)	Exposure time	Conditions and References*
Bean (*Phaseolus* spp)	9-35 (leaf)	0.1-10.0	3 wks	1
	1.8-19.0	2.5-160	seedling - maturity	5
	0.2-3.0 (seed)	0.1-10	3 wks	
Beet (*Beta vulgaris* L.)	280-1,078 (leaf)	0.1-10.0	3 wks	1
	0.5-2.5	0.1-1.0	5 wks	2
	30-51.8	2.1-5.6	5 months	3
Broccoli (*Brassica oleracea* L.)	1.3-8.8 (leaf)	2.1-5.6	5 months	3
Cabbage (*Brassica oleracea* var. *Capitata* L.)	212-822 (leaf)	0.1-10.0	3 wks	1
Carrot(*Daucus carota*L.)	0.2-2.2 (leaf)	0.01-1.00	5 wks	2
Celery (*Apium graveolens* L.)	5.83,14.87(stalk)	2.5,10.0	117 days	4
	3.77,10.77 (leaf)			
Lettuce (*Lactuca sativa* L.)	384-751 (leaf)	0.01-10.00	3 wks	1
	0.3-24.3	0.01-1	5 wks	2
	6.0-63.7	2.1-5.6	5 months	3
	11.5,27.1	2.5,10.0	37 days	4
(cv. Butter Crunch)	116.8-697.9	100-800	30 days	6
Head lettuce (*Lactuca sativus* L.)	5.6-41.6	2.1-5.6	5 months	3
Maize (*Zea mays* L.)	90-234 (leaf)	0.01-10.00	3 wks	1
	2.0-19.3	2.1-5.6	5 months	3
	11.0-94.0	2.5-160	seedling - maturity	5
	0.05-6.60 (grain)			
(cv. Dekalb Paolo)	9.7,21.1 (shoot)	0.01,0.05	15 days	10
	76.0,113.6 (root)			

Table 2.1 (continued)

Species	Cd accumulation (ppm)	Cd concentration in medium	Exposure time	Conditions and References
Pea (*Pisum sativum* cv. Argona)	10.1,24.1 (shoot) 63.8,117.8 (root)	0.01,0.05	15 days	10
	24.2-168.0 (shoot) 271.2-1,678.5 (root) 35.0-515.2 (nodule)	0.5-6.0 (perlite)	4 wks	7
	2.8,6.0 (shoot) 63.6,112.9 (root)	0.5,1.5 (vermiculite)	3-4 wks	7
Green Pepper	107-578 (leaf)	0.1-10.0	3 wks	1
(*Capsicum frutescens* L.)	3.82-6.28	2.5-10.0	112 days	4
Bell pepper (*C. annuum* L.)	5.9-26.2	2.1-5.6	5 months	3
Potato (*Solanum tuberosum* L.)	2.5-15.6 (leaf)	2.1-5.6	5 months	3
Radish (*Raphanus sativus* L.)	0.3-144.2 (leaf)	0.01-1.0	5 wks	2
	4.0-19.6	2.1-5.6	5 months	3
	10.20,16.13 (top) 4.2,5.93 (root)	2.5,10.0	26 days	4
(cv. Cherry Belle)	93.6-595.9 (root)	100-800	30 days	6
Rice (*Oryza sativa* L.)	0.1-0.7 (leaf) 0.1-1.1 (grain)	2.5-160	Seedling – maturity	5
Soybean (*Glycine max* Merr.)	3.7-35.0 (leaf) 3.9-29.0 (grain)	2.5-160	Seedling – maturity	5
(cv. Illini) (cv. Richland)	2234,164,17.4* 2194,78.3,10.4* *(root,stem,leaf)	44.48	6 days	8

Table 2.1 (continued)

Species	Cd accumulation (ppm)	Cd concentration in medium	Exposure time	Conditions and References
Swiss chard (*Beta vulgaris* L.) (subsp. *Clica*)	0.2-150.0 (leaf) 2.0-37.9	0.01-1.0 2.1-5.6	5 wks 5 months	2 3
Sunflower (*Helianthus annuus* L.)	114.0,2664.4(root)* 2.7,18.4 (stem) 0.4,1.2 (stem) *(μM/100 gDw)	0.009,88.97	2 days	9
Tomato (*Lycospersicon esculentum* Mill.)	58-1,122 (leaf) 0.3-158.0 5.0-16.5	0.1-10.0 0.01-1.00 2.1-5.6	3 wks 2 wks 5 months	1 2 3
Turnip (*Brassica rapa* L.)	160-469 (leaf)	0.1-10.0	3 wks	1
Wheat (*Triticum aestivum* L.)	1.2-9.0 (leaf) 2.2-48.0 1.5-14.0 (grain)	2.1-5.6 2.5-160.0	5 months seedling - maturity	3 5

* 1 nutrient solution (Page, Bingham and Nelson, 1972)

2 ¼ Hoagland's solution (Turner, 1973)

3 sludge-treated soil and contaminated soil (Kim et al., 1988)

4 Silty clay loam (Haghiri, 1973)

5 soil +sewage sludge+$CdSO_4$ (Bingham et al., 1975)

6 soil irrigated daily with 250 ml of nutrient solution containing Cd and 100-1000 ppm Pb (Nwosu, Harding and Linder, 1995)

7 perlite and vermiculite with nutrient solution (Hernandez, Gorate and Carpena-Ruiz, 1995)

8 modified Hoagland's solution with $Cd(NO_3)_2$ (Marchiol et al., 1996)

9 Hoagland's solution (Kastori, Petrovic and Petrovic, 1992)

10 nutrient solution (Lozano-Rodriguez et al., 1997)

2.4 Phytoremediation : an alternative method to ameliorate the Cd-polluted soil

Different decontamination or reclamation procedures have been suggested to minimized the metals hazard to plants, animals and humans and to restore the soil fertility for agricultural plants. The first method is to reduce bioavailability through addition of a nontoxic compound such as lime, iron sulphate, cation exchangers organic matter or clay such as bentonite. The second method attempts to dilute the contaminants in the uppermost soil layer by a deep plow down to 80 cm. The third method suggests the replacement of the upper soil layer with unpolluted soil. Another method is to reduce the metal by elimination. The results of the study in the site contaminated from brass foundry in Switzerland by using these methods have shown that only the replacement of the highly polluted soil leads to good germination, normal development and metal content in plant below the toxicity limits for Cd, Cu and Zn. The other procedures are not suitable for this site. Plowing the site distributes the highly contaminated interface-layer into the soil and the metals could be mobilized in the future. Decomposition of the contaminated organic matter would lead to a decrease of the binding sites and thus lead to a heavy metals release. Elimination of the litter leads to some reduction of the metal concentrations, but at the same time it may lead to the loss of the topsoil. Addition of the exchange resin reduced cation availability can lead to nutrient deficiency (Geiger, Federer and Sticher, 1993). Other method such as leaching with acids or chelates may have a risk that the amount of the bioavailable Cd may increase even though the total Cd content has decreased (Alloway, 1990).

Driel et al.(1995) investigated the thickness of non-polluted top soil required either to comply with the permissible levels for metal concentrations or to exclude any effect on plant metal levels. Protection of all food crops tested against exceeding permissible levels for Cd requires a clean top soil of over 1.6 m; for vegetable crops ranging from zero (no cover layer required for red cabbage, leek, onion and potato) to 1.2-1.6 m for celery tuber and leaf, for maize ranging from 0.25-1.20 m, and for wheat straw ranging from 0.55 to 1.60 m.

The remediation of large volumes of such soil by conventional technologies previously developed for small, heavily contaminated sites would be very expensive. It often costs between \$50 and \$500 per ton. Certain specialized techniques can exceed costs of \$1000 per ton. Recently, phytoremediation has emerged as an alternative to the conventional method. In this new approach, plants are used to absorb contaminants from the soil and translocate them to the shoot. Pollutants are then removed by harvesting the above ground tissue. The harvested biomass could be reduced in volume and/or weight by thermal, microbial, physical, or chemical means. This step would decrease handling, processing, and potential subsequent landfilling costs. The value of the reclaimed metal may provide an additional incentive for remediation (Cunningham and Ow, 1996).

It appears that the genetic potential exists for phytoremediation to be successful. A small number of plant species known as hyperaccumulators have been identified that are not only capable of growing on soil containing high levels of metals , but also accumulating those pollutants to high concentrations in the shoots. The well known species, for instance, is *Thlaspi caerulescens* J. & C. Presl, a Zn and Cd hyperaccumulator. This plant grown hydroponically could accumulate in shoots up to 32,500 mgZn/kgDw and 1,270 mgCd/kgDw from a solution containing 10,000 μM Zn and 200 μM Cd. Concentrations of Zn in harvestable shoots are high enough that plant tissue could be treated as a low grade ore. Zinc and Cd could be recycled by smelting dried shoots (Brown et al., 1995).

However, low yield and slow growth rate are the two major factors that limit for the potential for the phytoremediation of Zn/Cd contaminated soils by successive croppings of *T. caerulescens*. In addition, it is a low-growing plant, which makes any mechanical harvesting problematic (Brown et al. , 1995). Recent study has shown that three *Brassica spp.-B. juncea,* (L.)Czern *B. napus* L. and *B. rapa* L.- are more effective in removing Zn from soil than *T. caerulescens*, whereas Cd removal is comparable for *T. caerulescens*. This is because the *Brassica* spp. produced more than ten times the shoot biomass produced by *T. caerulescens* (Ebb et al., 1997).