A BIOPHYSICALLY BASED FRAMEWORK FOR EXAMINING PHYTOREMEDIATION STRATEGIES: OPTIMIZATION OF UPTAKE, TRANSPORT AND STORAGE OF CADMIUM IN ALPINE PENNYCRESS (THLASPI CAERULESCNES)

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TERMINOLOGY

Compartment	Major storage unit considered in the model (i.e. root, stem, leaf), which includes both the portion floating in the cytoplasm as well as part that is bound to cell walls and vacuoles
Cytoplasm	Liquid that fills the plant cells
Cell walls/ vacuoles	Surface area to which the Cd can be bound within a compartment
Order of uptake	The assumption that the Cd is taken from the soil into the roots, then stem and then into the leaf
Regional gain	Input of Cd from the preceding compartment into the target compartment in its floating form (cytoplasm)
Local "loss	Input from cytoplasm form to bound form
Regional loss	Output from cytoplasm of the target compartment into the following compartment; this process does not exist for the leaf compartment
Xylem	Also known as the "highway" of the plant, this structure allows advective transport of the Cd from one compartment into another. Responsible for the transport of water and mineral nutrients from the roots throughout the plant

LIST OF ABBREVIATIONS

Parameters

α	Uptake rate
β	Surface-to-biomass ratio
K	Half saturation constant if K_m; otherwise, permeability constant (described later)
k	Growth rate
i	Ratio increment rate

Variables

C	Concentration
M	Mass of Cd
B	Biomass of a particular compartment (such as root)
R	Ratio between the compartments

Descriptions (subscript, superscript)

*	Partial expression (e.g. not necessarily explaining the entire uptake process)
t	current time (if a variable is not specified, it's the current time by default)
t+1	one step in the future
ave	average of two time steps, namely *t* and *t+1*

r	root compartment
s	stem compartment
l	leaf compartment
c	Cd floating in the cytoplasm
b	Cd bound to the cell walls/vacuoles
min	minimum or threshold concentration before there is net uptake
max	phytotoxicity limit
m	half saturation constant for root uptake
f	final (as in final weight, if M_f)

CHAPTER I

SOIL CONTAMINATION AND REMEDIATION STRATEGIES

Introduction

As societies increasingly rely on manufactured chemicals, soil contamination can become a major public health concern. From organics to radionuclides to heavy metals, soil contamination arises from accidental spills from engineered structures, application of pesticides and herbicides, percolation of contaminated surface water to subsurface strata, leaching of wastes from landfills, and direct discharge of industrial wastes to soils (McGrath *et al*., 2002; Adriano, 2001). More than 200,000 sites in the U.S. are identified for soil contamination. Yet until the 1970s, there was limited awareness of the dangers associated with soil contamination. In the 1980s, the U.S. Environmental Protection Agency (USEPA) established regulatory standards for soil quality. However, because contaminated soil sites are wide-spread and large in volume, and because of the extent and difficulties of managing contaminated soils, billions of dollars in costs can depend on the selection and implementation of appropriate remedial decisions.

Cadmium (Cd) is a naturally occurring transition metal element found in almost all environmental media, and in the II oxidation state naturally (Adriano, 2001). In non-polluted areas, sedimentary rocks typically contain the highest concentrations of about 3.5 parts per

million (ppm). Cd tends to be stable in the divalent form although the most common Cd compound in nature is CdS. Until its toxicity became apparent in 1960s, the exposure to Cd was relatively high in developed countries, especially in the United States, Japan and the former Soviet Union, due to its usage in factories. Cd is produced commercially as a by-product of the Zn industry. In addition to Cd, literature findings regarding Zn are discussed in this thesis due their chemical similarities. Although there are a limited number of commercial applications of Cd, its usage is wide-spread: protective plating for steel, stabilizers for polyvinyl chloride, pigments in plastics and glasses, electrode material in nickel-cadmium batteries, and as a component of various alloys (Friberg *et al*., 1992; Adriano, 2001). Therefore, Cd can be found in household appliances, automobiles and trucks, agricultural implements such as phosphorous fertilizers, airplane parts, industrial tools, and hand tools, and fasteners of all kinds are commonly cadmium-coated (Kirkham. 2006; Lasat, 2000).

Humans can directly ingest Cd from food, liquids or edible products that are in contact with Cd-coated containers. Cd is taken up through the roots of plants to become concentrated in edible leaves, fruits and seeds; this biological phenomenon is the basis of this study. Cd also builds up in animal milk and fatty tissues. Seafood, such as mollusks and crustaceans, can be a source of cadmium as well (Kaneta *et al*., 1986).

Cd was the cause of Itai-itai disease in Japan during the 1950s, where ingestion of the

metal led to brittle bones and kidney failure along with other side effects. Because of this incidence, the Japanese government has set 1 ppm of Cd in rice grain as the maximum allowable limit, as opposed to 3.4 ppm, which was commonly found during the incident (Kaneta *et al*., 1986; Adriano, 2001). Oral exposure to Cd has adverse affects on the kidney, liver, bones, testes, the immune system, and the cardiovascular system. Beyond 20 mg Cd per kg of human tissue, excessive fluid loss may eventually lead to death (NIH, 1998; Lasat, 2000).

In an effort to protect people from exposure to Cd, various government agencies have taken steps to protect residents from Cd exposure. The USEPA controls the concentration of the discharge and solid wastes from factories; the Occupational Safety and Health Administration (OSHA) sets limits on maximum permissible exposure to Cd dust and fumes; and the Food and Drug Administration (FDA) controls the amount of Cd in ceramic plates (ATSDR, 1998).

Phytoremediation

Along with physical excavation, chemical barriers and bioremediation, phytoremediation is a technique used to isolate and clean up contaminated sites. Traditional treatment methods such as excavation require subsequent treatment and disposal of the removed soil and sediments. Furthermore, usually the pore solution contained in the sediment needs treatment

as well. In the US alone, the remediation of metal-contaminated soils based on relatively destructive engineering-based methods is estimated to cost about 400 billion dollars (Milner and Kochian, 2008).

As opposed to these costly invasive techniques, phytoremediation is gaining popularity as an eco-friendly and aesthetically-pleasing option. In some situations, the vegetation simultaneously stimulates bioremediation, a technique where microorganisms immobilize and/or chemically transform contaminants. The market in the US alone for the decontamination of soil and groundwater through phytoremediation was in excess of $100 million in the year 2000, and this technique is expanding rapidly (Fitter *et al.* 2002). Due to various capabilities for different species to stabilize, sequester, store, evaporate and/or transform the original contaminants into less harmful products, most soil contaminated by organics, radionuclides, metals are treatable through phytoremediation (Gouthu *et al*., 1997; Grispen *et al*., 2006; Pugh *et al*., 2002; Narayanan *et al*., 1999).

Among various phytoremediation techniques, phytoextraction, also known as phytoaccumulation, is the main subject of this study, and has been used as an effective method for cleaning up contaminated environments. The idea is to remove contaminants, specifically heavy metals or radionuclides, from a soil by utilizing the natural capability of plants to take up the contaminants along with water and essential nutrients and store them in aboveground tissues. Eventually the plants are harvested to be treated and/or disposed.

Secondary benefits of this method are decreased rates of transport of contaminant by wind, rain and groundwater due to physical (plant) obstruction and increased water evaporation (USEPA, 2001). Due to the ease of this technique, those plants designated to accumulate the contaminants are referred to as natural "vacuum cleaners". Specifically, the plants that favorably/selectively uptake and store unusually high amounts of the pollutants in the aboveground biomass are referred to as hyperaccumulators (USDA, 2007; Lasat, 2000). The criterion for defining hyperaccumulation is also 100 μg Cd per g in stem dry matter and 10,000μg Zn per g in stem dry matter. Although these criteria are somewhat arbitrary, the concentrations of metals in hyperaccumulators are about 100 to 1,000 fold higher than those in normal plants growing on soils with background metal concentrations and about 10 to 100 folds higher than most other plants growing on metal-contaminated soils (McGrath *et al*., 2002).

Thlaspi caerulescens, commonly known as alpine pennycress, has been studied extensively as a promising hyperaccumulator of Cd (Kirkham. 2006; Lasat, 2000; McGrath *et al.*, 2002) and is the subject of this study. Alpine pennycress is a small, weedy member of the broccoli and cabbage family that thrives on soil that contains high levels of zinc (Zn) and Cd (USDA., 2007). In fact, pennycress plants that were originally from regions with high Cd concentration in the soil had better growth --- by developing more and bigger leaves, taller stems, and by producing more fruits and heavier seeds --- when planted in slightly Cd

contaminated soil than non-polluted environment (Basic *et al.*, 2006).

Along with its tendency to favorably accumulate contaminants of interest, an effective hyperaccumulator should fulfill most, if not all, of the following requirements (Padmavathiamma *et al*., 2007; Grispen *et al*., 2006):

1. Sufficient tolerance for the contaminant of interest;
2. Absorb large quantities of toxin into the roots;
3. Transport the toxin to the stems;
4. Have a high growth rate; and
5. Minimize the risk of transferring metals to high trophic levels of the food chain.

First requirement may seem redundant as the unusually high tolerance of the plants to the specific contaminants is the definition of hyperaccumulators. Unlike nutrients such as nitrogen (N) or phosphorous (P), which allows greater biomass than in absence of those nutrients, Cd is a non-essential element where the acceptable concentration range is only between 0.05-2 ppm (Table 1.1; Blackshaw *et al*., 2004). Furthermore, the tolerance of this heavy metal can vary even among alpine pennycress and results in various plant health impacts (Roosens *et al*., 2003; Das *et al*., 1997). One measure of plant health is its biomass. Figure 1 gives a good illustration of the difference in biomass for a population of *Sedum alfredii*, another Zn/Cd hyperaccumulator commonly found in China discussed more in detail

later in this chapter, that is accustomed to heavy metal loading as opposed to an another population of *S.alfredi* that is not normally exposed to heavy metals (DeChamps *et al*. 2004). In addition to a decrease in biomass, the latter population also showed other phytotoxicity symptoms such as wilting, drooping leaves and root necrosis (Sun *et al*., 2007, Zhou and Qui, 2005, Xavier da Rosa Corre *et al.*, 2006; Das *et al*., 1997). In general, typical phytotoxicity effects can be summarized in five categories: modifications in the development cycle, thinning, modification in color, necrosis --- local death of tissues or organs --- and deformation (OEPP, 2007).

Table 1.1: Effect of typical levels of heavy metals in plants (Padmavathiamma *et al*., 2007)

Status	Metal concentrations (mg kg^{-1})			
	Cd	Cu	Pb	Zn
Deficient	–	<1–5	–	<10
Normal	0.05–2	3–30	0.5–10	10–150
Phytotoxic	5–700	20–100	30–300	>100

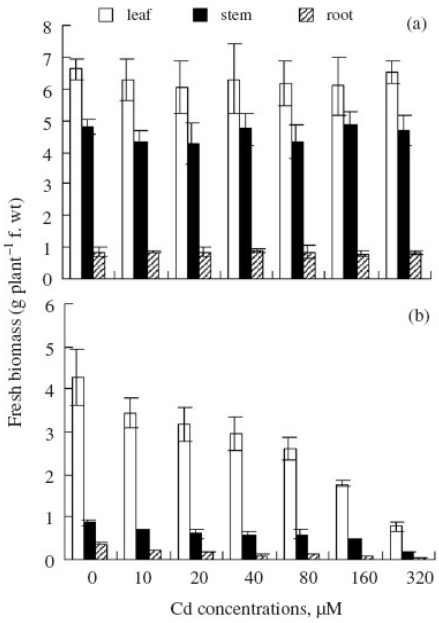

Figure 1.1: Fresh weights of different tissues of mine (a) and non-mine (b) populations of *Sedum alfredii* exposed to a range of Cd for 7 days ;mean +/- SD, n =3. (DeChamps *et al*. 2004)

The first requirement also involves a second important idea pertaining to changes in plant tolerance at different stages of growth. A plant may be tolerant of high levels of Cd or Zn when it is transplanted to contaminated soil after growing to maturity in an uncontaminated soil, but it may not germinate if the seeds are placed in a contaminated soil.

Even if the level of the contaminant of interest may not pose a problem, if the soil mixture also contained other contaminants that inhibit the growth of the plant, then its remediation capacity is limited. For an example, alpine pennycress has limited tolerance for copper (Cu). Therefore, despite its excellent remediation capability for Cd and Zn, this plant may not be able to accumulate these transition metals if the Cu toxicity inhibit normal functioning of the plant (McGrath *et al*., 2001).

Alpine pennycress is an effective Cd hyperaccumulator, fulfilling the second requirement. Sometimes hyperaccumulators can contain even higher concentrations of the metal than that of the soil (Lagerwerff *et al*., 1971). According to the study from Lombi *et al*. (2001b), over a period of 391 days, alpine pennycress were found to contain up to 263 mg of Cd per kg of biomass, which is much greater than 100 mg/kg of biomass, the typical toxicity limit for alpine pennycress defined by most authors. Furthermore, another study showed that alpine pennycress grown in a nutrient + Cd solution may accumulate up to 10,000 mg of Cd per kg of fresh biomass before phytotoxicity symptoms appear, though uptake is typically one to two orders of magnitude lower when grown in soil (Maxted *et al*. 2007).

Whereas most plants avoid absorption of heavy metals, it may seem odd for pennycress and other hyperaccumulators to purposefully take up and store heavy metals in its tissues (Figure 2). One evolutionary theory that attempts to explain this rare phenomenon states that the metal accumulation is a self defense strategy – leaf feeding insects are discouraged from eating the leaves when the plant contains Zn and/or Cd (Jiang *et al*., 2005). Another explanation states that the Zn transporters, proteins required at the root membrane for uptake, also have some affinity to Cd, therefore transporting the Cd in the process of transporting Zn, an essential element (Maxted *et al*. 2007, Schwartz *et al.*, 2003, Zhao *et al*., 2001; Lombi *et al*, 2001a).

Regarding requirement 3, the capability to transport a contaminant from the root to the

stems is probably the most important characteristics of a hyperaccumulator (Xing *et al*., 2008). A typical plant stem Cd concentration is 0.1 - 10 ppm, whereas that of an alpine pennycress is as much as 10,000 ppm (Milner and Kochian. 2008). After all, if the heavy metals are not stored in the aboveground tissues, the contaminant cannot be harvested with the biomass.

Interestingly, when Zn and Cd uptake were compared between alpine pennycress and a related non-accumulator, field pennycress (*Thalspi arvense*), most Zn and Cd was stored in roots for field pennycress with almost no stem metal concentration, whereas alpine pennycress translocated significant amounts of Zn and Cd in the stems (Ozturk *et al*., 2003; Lasat *et al.*, 1996; Lasat *et al*., 1998; Xing *et al*., 2008; Sun *et al*., 2007).

The fourth idealistic feature is a large biomass. The efficiency of phytoextraction is the product of a simple equation: biomass x element concentration in biomass. Unfortunately, pennycress weighs less than 10 grams. This raises the following question: If there is no hyperaccumulator that has large biomass, could a regular plant with high biomass that is capable of taking up contaminants, albeit not at the rate of hyperaccumulators, clean up a site just as effectively? A study from Lombi *et al*. (2001b) compared chemically enhanced phytoextraction by maize (*Zea mays*) of Cd + EDTA (ethylene-diamine-tetraacetic acid), a chelating agent that increases the bioavailability of Cd in soil, to that of the phytoaccumulation done by pennycress for untreated Cd. In this study, most of the metal in the maize was stored in the roots rather than stems, failing the third criteria for

hyperacumulator, therefore phytoextraction would not be an effective means of remediation (Das *et al*., 1997). To worsen the situation even more, what remains in the roots is released to the soil with decomposition of the plant matter, thereby contributing to a temporal spike in the heavy metal concentration in the soil solution. Furthermore, by adding chelating agents, the mobility and bioavailability of Cd increases, therefore transport through percolation down through soil medium also becomes easier (Wenzel *et al*., 2003). Because the tolerance level of the maize is much lower than that of the alpine pennycress, they would need to be harvested immediately after the chemical treatment, putting strict schedule constraints on harvestation (Kirkham 2006; Macnair, 2003). Therefore even though many hyperaccumulators are small, the difference in concentrations that the hyperaccumulators can tolerate and non-accumulator high biomass crop is often greater by 100 to 1,000-folds, whereas the difference in biomass is most likely to be in the order of magnitude of two, therefore the most limiting factor for present phytoextraction technology is no the biomass (McGrath *et al*., 2001).

Lastly, but not least, minimizing the risk of transferring metals from alpine pennycress to higher trophic levels of the terrestrial food chain is a management issue that needs to considered when implementing this technology. Where alpine pennycress is naturally occurring, it is subject to livestock grazing (Montana Field Guide, 2008; Raskins *et al*., 1994). Therefore, transfer into other organisms is another concern that phytoremediation in general introduces.

As an alternative to the alpine pennycress, *Sedum alfredii* is reported as Zn/Cd hyperaccumulator for the first time in 2002. Its features like fast growth, large biomass, asexual reproduction and perennial growing up to 40 cm in height, propagate 3-4 times in a year under favorable environmental conditions make this species worth examining. Also the max Cd concentrations in stem and leaf is 4512 mg of Cd per kg of plant biomass as opposed to 100 mg Cd kg^{-1} for pennycress (Sun *et al.* 2007). The only drawback in implementing this plant for phytoextraction purposes in the U.S may be the fact that this Chinese native plant may not be favorably accepted over endemic alpine pennycress.

Choosing phytoremediation over conventional invasive approaches as a clean-up technique seems appropriate for mild to moderately polluted soil (Macnair, 2003; Maxted *et al*., 2007). Based on a literature review, alpine pennycress possesses many of the important characteristics of an ideal phytoextracting plant. Although the biomass is small, looking at long term management issues such as harvesting and planting frequency, there is merit to examine alpine pennycress (Robinson, 1998).

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CHAPTER II

PHISIOLOGY OF METAL HYPERACCUMULATION

Introduction

Whenever plants grow on contaminated soil, pollutants may be incorporated within the aboveground tissue of the plants. Currently, scientists know about 400 plant species, less than 0.2% of all angiosperms, in families such as *Asteraceace*, *Brassicaceae*, *Caryophyllaceae*, *Poaceae*, *Violaceae* and *Fabaceae* that possess both the ability to uptake high amounts of heavy metals and store them in stems (Adriano, 2002; Ghosh and Singh,2005). The *Brassicaceae* is best represented and known to have 87 species that are classified as metal hyperaccumulators, one of which is alpine pennycress *T.caerulescens* (McGrath *et al*., 2002; Grispen *et al*., 2006; Raskins *et al*., 1994). In order to ensure a successful phytoextraction practice, the complex interaction between climatic conditions, soil properties, site hydro-geology as well as basic plant mechanism should be modeled (Lasat, 2000).

This chapter covers both qualitative and quantitative descriptions of uptake, transport and storage of heavy metals as these pollutants journey from soil to epidermal cells in leaves. The discussion also extends to the general growth behavior of plants, a concept incorporated in one of the model simulations in the next chapter. The ultimate aim of this

study is to mathematically simulate fundamental physiological and biochemical differences between the hyperaccumulators and non-accumulators.

Cd availability in the soil

In order to estimate the uptake of metals by roots, a model needs to consider heavy metal concentrations in the soil, root sizes and surface area, and inflow into the roots (Clarkson, 1985). The problem basically hinges on supply of the metals, the plants' demand for the metals, and the uptake mechanism.

Generally, Cd is fairly immobile in the soil profile, evidenced by both unpolluted and polluted soils. When Cd is found in uncontaminated soil, it usually retains the Cd concentration of the original parent rock unless the soil has been subjected to long-term cultivation. Cd retention is also high, as evidenced by a soil that contained 90% of the Cd applied as a sewage sludge mixture in the top 0 - 15 cm of the surface, equivalent to the depth of accumulation over the years of application (Adriano, 2001). Ironically, in another case, Cd arising as a side product of P fertilization resided much longer than the P itself (Adriano, 2001; McGrath *et al*. 2002). From an engineering standpoint, this relative immobility in soils is favorable for clean up.

Total Cd content in soil is important to preliminary assess the potential hazard of the area. However, the bioavailability of the Cd is even more important parameter when considering

the metal in the context of phytoextraction. Definition of the availability goes as "the rate and extent at which a chemical is released from a medium of concern or the bioavailability of the chemical to living receptors" (Kirkham, 2006). Most labile form of the metal are found in the soil solution as free metal ions and soluble metal complexes or adsorbed to inorganic soil constituents at ion exchange sites (E value in Figure 2.1; Lombi *et al*. 2001b); however, there are theories that a plant may also access non-labile or "fixed" form, such as Cd bound to soil organic matter, precipitated such as oxides, hydroxides, and carbonates; and embedded in structure of the silicate minerals (L value in Figure 2.1; Lasat, 2000; Raskins *et al*., 1994).

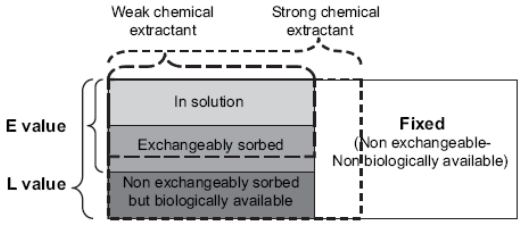

Figure 2.1: Schematic representation of the definition of the E and L values and the extraction power of weak and strong chemical extractants. The size of the boxes is not at scale (Hammer *et al*., 2006).

This topic begs the question whether or not the hyperaccumulator has greater capacity of immobilizing the Cd into bioavailable form than the non-accumulators, therefore resulting in higher uptake of the metals. Some previous studies suggested that the hyperaccumulator may release some of the immobile forms of Cd through root exudate. However, that does not seem to be the case; between alpine pennycress and *Brassica napus*, a

non-accumulator species, L value test showed that despite taking up more metals, pennycress accessed the same pool of metals as *B.napus* (Hammer *et al*. 2006; Ayoub *et al*. 2007; Maxted *et al*. 2007; Knight *et al*. 1997). Also in hydroponic studies, where the soluble and bioavailable Cd concentration is the same, the uptake rate of the hyperaccumulator is still significantly higher (Lasat, 1996). This finding suggests that the differences in the uptake rate must be explained by the uptake mechanism rather than availability.

pH, cation exchange capacity (CEC), redox potential as well as presence of organic matter, other chemically similar metals, and fertilizers govern the bioavailability. Cd, like all other heavy metals, is almost always more extractable with decreasing pH (Everhart, 2006; Yanai *et al*. 2005). Metal desportion from soil binding sites into solution is stimulated due to H^+ competition for binding sites (Lasat, 2000). As long as the plant itself does not suffer damage from low pH, the Cd absorption by lettuce, Swiss chard, radish, corn and rice, to name a few, has been greater in acidic conditions (Adriano, 2001). Also related to pH is CEC, a capacity of a soil for ion exchange of positively charged ions between the soil and the soil solution. High CEC provides a buffering effect due to greater sorption and immobilization capacity of the metals. In addition, especially in unpolluted soils, CEC is inversely correlated with Cd bioavailability (Adriano, 2001; Lasat, 2000).

Other metals such as Zn, Ca, Mn in solution seem to compete with Cd. Therefore, even if Cd surrounding a root is in readily available form, the uptake may be inhibited if other metals

are also in abundance. Because Cd competes with Zn in forming protein complexes at the uptake sites, the presence of one metal specie can negatively affecting the total uptake of the other can be expected (Cosio *et al*., 2004; Milner *et al*., 2008).

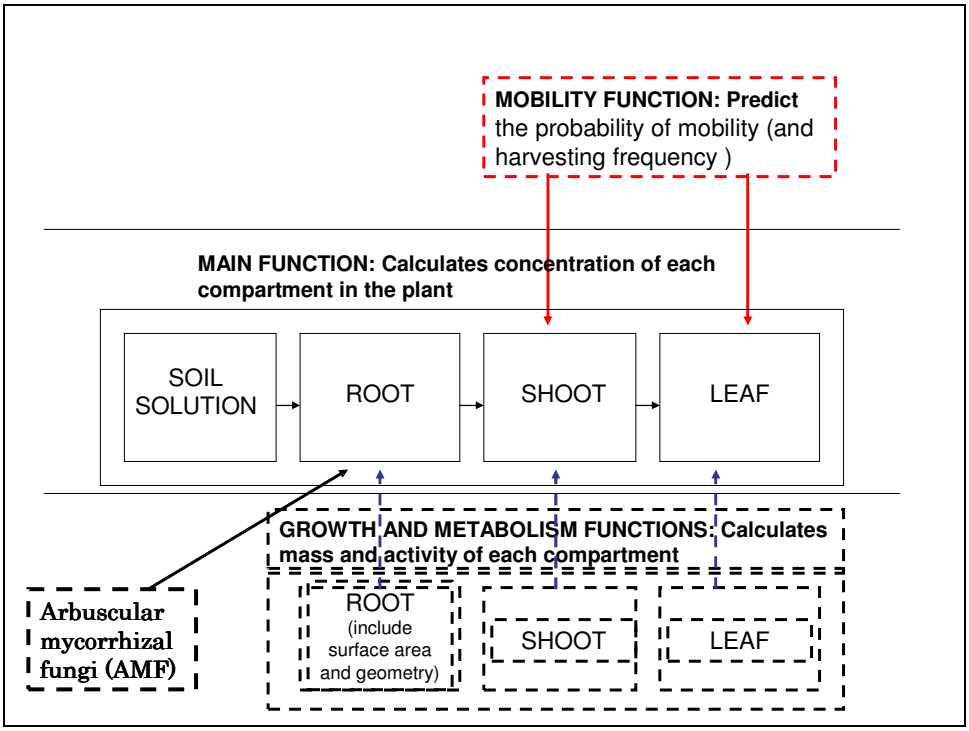

Figure 2.2: Schematic for model

Roots: Uptake, Transport and Storage

Hyperaccumulators tend to strongly localize their roots where high concentrations of heavy metals occur, that is, within "hot spots" when the concentration distribution is uneven. In contrast, a non-accumulator has an opposite behavior, avoiding Cd-rich soil hot spots (Vogel-Mikus *et al*. 2005; Schwartz *et al*. 2003; Hammer *et al*. 2006). Once the roots are

densely grown, they may compete for bioavailable metals as the radii of influence surrounding roots start to overlap with one another. Models that make no allowance for root competition tend to overestimate the uptake of mobile nutrients such as potassium (K), while still giving reasonable fits for Cd uptake, which is immobile in nature (Clarkson, 1985). Regardless of the competitive uptake between the roots, a hyperaccumulator's preference for heavily metal-contaminated soil, as opposed to non-accumulator that avoids those hot spots, results in higher metal tissue concentration in the hyperaccumulator over non-accumulator (Vogel-Mikus *et al*. 2005).

Unlike passive nutrient uptake, specific transporters on root membranes are required for active Cd uptake. At the molecular scale, high levels of the Zn transporter, *ZNT1*, which is primarily responsible for Zn intake, but also has some affinity for Cd, are expressed in hyperaccumulators even when the plants have high Zn tissue concentration. Non-accumulators such as field pennycress show much weaker expression of the protein and the expression was stimulated by Zn deficiency (Pence *et al*. 2000; McGrath *et al*., 2002). The difference in the abundance of the transporters would partially explain why the hyperaccumulators would higher Zn concentration. In the case of Cd, its uptake is believed to be unspecific and inadvertent via transporters for other essential nutrients such as Fe, Zn, and Mn, although some recent studies also hypothesize the existence of Cd specific transporters (McGrath *et al*, 2002; Lombi *et al*., 2001a).

Most terrestrial plants have a limited capacity for dealing with excess metals, and store these heavy metals in the cell walls and vacuoles in order to lower concentration in the root cytoplasm, and to prevent transport into the stems so as to minimize damage to the photosynthetic apparatus (Clemens, 2000; Clemens *et al*., 2002; Milner and Kochian. 2008). *ZTP1* is a transporter found on tonoplast, the cytoplasmic membrane surrounding the vacuole, separating the vacuolar contents from the cytoplasm in the cell, especially concentrated in the leaves and is also believed to minimize damage to the photosynthetic function (Milner and Kochian, 2008). *HMA*, heavy metal ATP-ase, primarily found in roots, is another player believed to increase Cd tolerance and is responsible for transport into the xylem leading to further transport into aboveground biomass.(Milner and Kochian. 2008; White *et al*., 2002; Pence *et al*. 2000). Aside from the removal through vacuole binding, although not discussed in depth in this thesis, complexation of metals with ligands results in decreased free ion activity and thus decreases toxicity (Clemens, 2000; Clemens *et al*., 2002; McGrath *et al*., 2002; Zhang *et al*., 2008; Sun *et al*., 2005).

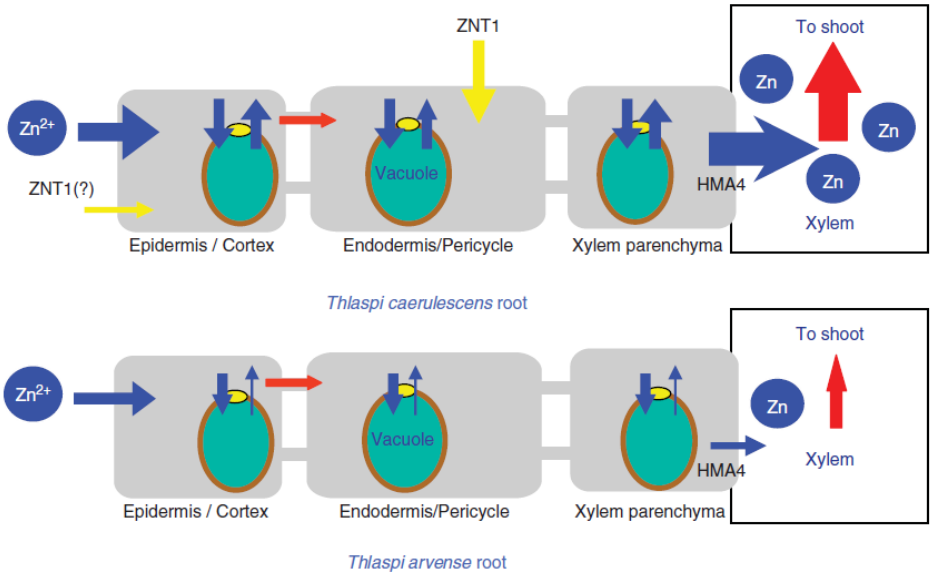

Figure 2.3: Model of Zn transport into and across the root pennycress. The model depicts some of the aspects of and differences in the movement of Zn^{2+} from the soil into and across the root of hyperaccumulating alpine pennycress (*T.caerulescens*) and non-accumulator field pennycress (*T. arvense*). (Milner and Kochian, 2008).

It is noteworthy that there are evidences of plants forming possible symbiotic relationship with arbuscular mycorrhizal fungi (AMF). AMF associations increase the area of nutrient exploitation in soil by roots and usually increase acquisition of nutrients in nutrient limiting conditions (McGrath *et al*., 2002; Sung *et al*., 2001). This has triggered several studies that explore the contribution of fungi to plant uptake; however, results are mixed on whether or not they are effective at either inhibiting or enhancing heavy metal uptake. Members of the *Brassicaceae* are generally believed to be non-mycorrhizal and despite the diverse samples of pennycress from Slovenia, Austria, Italy and Germany, at best, these plants were poorly colonized. Therefore the chance is small to find these organisms actually aiding effectiveness of phytoremediation using pennycress (Regvar *et al*. 2003).

Root's Regional Loss: Soil solution to root cytoplasm

Knowing that Cd inflow to the root is a function of the concentration of Cd in the soil solution and the concentration of Cd in the root cytoplasm, the following describes the uptake of Cd into the root:

$$\frac{dC_{r,c}}{dt} = \alpha_{r,c} = \alpha_{r,c,\max}\left[\frac{(C_{sol} - C_{sol,\min})}{K_m + (C_{sol} - C_{sol,\min})}\right]^a \left[1 - \frac{C_{r,c}}{C_{r,c,\max}}\right]^b \quad (1)$$

Here, concentration of Cd 'floating' in the root cytoplasm increases according to a Michaelis-Menten-type uptake, which is only a function of the soil solution concentration (Grafe and Kuchenbuch, 2002). $\alpha_{r,c,\max}$ is the fastest possible uptake rate, specifically governed by the number of active binding/transport sites. If the soil solution concentration C_{sol} is high, there is plenty of Cd in the soil solution for the root to take up; therefore the actual uptake rate $\alpha_{r,c}$ approaches $\alpha_{r,c,\max}$. In order to account for competition of Zn, Mn or Ca with Cd for binding sites, $\alpha_{r,c,\max}$ would need to be reduced accordingly (Das *et al*., 1997; Macnair, 2003).

$C_{sol,\min}$ in (1) describes the lowest concentration that needs to be achieved before net uptake by the root occurs. Below this minimum concentration, the probability of Cd encountering a binding site is negligible, and therefore no uptake is observed. It is important to note that negative uptake is not physically possible, therefore (1) is applied only when C_{sol} is greater than $C_{sol,\min}$. Since hyperaccumulator has more densely packed uptake sites than non-accumulator, increasing the chance of uptake, alpine pennycress would have lower $C_{sol,\min}$ than field pennycress.

The half-saturation constant K_m, the concentration at which the uptake rate is half of the maximum uptake rate, controls the uptake rate. K_m represents the affinity of the transporter to Cd. A low K_m value, high affinity, indicates that high levels of ions are transported into the cells, even at low external ion concentration (Milner and Kochian. 2008; Lasat, 2000).

In addition, the uptake process may be slowed or completely shut down as the root cytoplasm approaches the phytotoxicity limit $C_{r,c,max}$ due to enzyme degeneration that Cd causes. The exponents *a* and *b* modulates the strength of how much the uptake and the counter-acting regulatory mechanism affect the overall uptake rate, respectively. The *a* to *b* ratio is expected to be greater for a hyperaccumulator than for a non-accumulator because non-accumulators react quickly to avoid intake of Cd once the Cd level is even slightly elevated, therefore making *b* for a non-accumulator a greater value than for a hyperaccumulator.

Root's Local "Loss": cytoplasm to vacuole and cell wall exchange

Once Cd is inside the plant, it can either remain in the cytoplasm, go into vacuoles, or be transferred into the xylem. From a floating Cd molecule in root cytoplasm to being bound to cell walls/vacuoles in the root is described as:

$$\alpha_{r,b} = \alpha_{r,b,\max}\left[\frac{C_{r,c}}{K_r + C_{r,c}}\right]^c\left[1 - \frac{C_{r,b}}{C_{r,b,\max}}\right]^d \quad (2)$$

Note that this is a “loss” from the cytoplasm standpoint, and conversely a “gain” for Cd bound in the roots. The form of the equation is very similar to that of the regional gain. The Michaelis-Menten-like uptake is regulated by how permeable K_r is, or in other words, how easily accessible the cell wall/vacuole binding sites are for the floating Cd and may be a function of *ZTP1* abundance, analogous to *ZNT1* affinity to Cd. $\alpha_{r,b,\max}$ describes the fastest rate at which the Cd can bind to these available sites.

The second parenthetical portion of (2) describes the availability of the binding sites. As the availability diminishes, the process is slowed and eventually ceases. The exponents *c* and *d* describe the extent of influence that accessibility and vacancy of binding sites have on the overall binding rate, respectively. The available binding sites are function of vacuole surface area to root biomass ratio β_r, the average root biomass over the time difference B_r^{ave}, and the actual binding rate $\alpha_{r,b}$ from equation (2):

$$\frac{dM_{r,b}}{dt} = \beta_r B_r^{ave} \alpha_{r,b} \tag{3a}$$

where the concentration C_r is obtained by dividing the mass of Cd by the total compartment biomass, or

$$C_r = \frac{M_{r,b}}{B_r} \tag{3b}$$

Root's Regional Loss: Going from root cytoplasm to stem cytoplasm

The advective regional loss of the root cytoplasm through xylem driven by transpiration is also the regional gain of the stem cytoplasm (Appendix A). Therefore the higher the $C_{r,c}$, the more it is advected upwards into the next compartments but also counteracted by the phytotoicity limit of the stem, as described below:

$$\frac{dC_{s,c}}{dt} = \alpha_{s,c} = \alpha_{s,c,\max}(C_{r,c} - C_{r,c,\min})^{e}\left[1 - \frac{C_{s,c}}{C_{s,c,\max}}\right]^{f} \quad (4)$$

The above equation only applies when $C_{r,c}$ is greater than $C_{r,c,\min,}$ or else the transport of Cd to the stem is negligible. $\alpha_{s,c,\max}$ is mainly a function of the transpiration rate and the ease with which it reaches the xylem, related to the *HMA* abundance.

The expression in the second parenthesis in (4) describes the slowing of the process as the stem cytoplasm reaches its toxicity limit, analogous to the toxicity limit of the root. The exponents *e* and *f* describe the extent of advective transport and how the shut-down mechanism affects the overall uptake rate, respectively.

Mass Balance in the Root Compartment

In summary, a change in the concentration of Cd in the root cytoplasm is equal to the inflow from the soil solution (1) minus the amount lost to vacuoles within the roots (2) and to xylem that transports Cd to the stems and leaves (4), or mathematically described as: .

$$\frac{dC_{r,c}}{dt} = \alpha_{r,c} - \frac{\beta_r B_r^{ave} \alpha_{r,b}}{B_r} - \alpha_{s,c} \quad (5)$$

For Cd bound to the roots, the change observed is solely a function of the local "gain". As discussed previously, if there are vacancies in the vacuole, there is inflow into the vacuoles. If this is a net gain for vacuoles, the change in concentration with respect to time is positive.

$$\frac{dC_{r,b}}{dt} = \frac{\beta_r B_r^{ave} \alpha_{r,b}}{B_r} \quad (6)$$

Stems and Leaves: Transport and Storage

Regional gain and loss : Transpiration driven transport

The *regional loss* for a root is that amount of Cd advected into the stem cytoplasm using xylem, also known as *regional gain*. Analogously, the *regional loss* for the stem becomes a *regional gain* of leaf cytoplasm. Because Cd is not volatile, there is no regional loss for the leaves, and therefore any Cd reaching the leaves will permanently stay in the compartment.

Important factors influencing final leaf concentrations could be activity levels of metal transporters, as mentioned previously, as well as transpiration rates (Macnair, 2003). The magnitude of these fluxes must be significantly larger for hyperaccumulators relative to non-accumulators for them to have great upward transport capacity evidenced in the literature findings.

Local "loss": Sequestration at Midway and the Final Destination

Provided that all the cell walls and vacuoles are not saturated, the Cd in stem is either stored sequestered in the stem either as a bound Cd to vacuole or a ligand complex or travel up to the leaf. As for the Cd that reached the leaves, epidermal cells, which contain no choloroplast, have 2-fold higher concentration of Cd than in mesophyll cells, which are primarily responsible for carrying out phytosynthetic operations(Cosio *et al*., 2005; Ma *et al.*, 2004; McGrath *et al*., 2002).

Growth of the Plant using Logistic Model

Growth of the plant ties directly to the concentration of Cd because the amount of Cd is divided by the biomass as described in equation (3b). The model in this study uses logistic equation to describe the growth of the root and stem biomass combined (Caloin and Yu, 1982). The rate of growth in mass is a product of growth coefficient *k*, the current mass M_{r+s}., and the difference between the final and current masses, $M_{r+s}/M_{r+s,f}$, as described below:

$$\frac{dM_{r+s}}{dt} = kM_{r+s}(1 - \frac{M_{r+s}}{M_{r+s,f}}) \quad (7)$$

where combined biomass M_{r+s} is a summation of root biomass M_r and stem biomass M_s, or

$$M_{r+s} = M_r + M_s \quad (8)$$

Similarly, the stem-to-root ratio is a homologous function starting from zero, meaning no stem is present initially, and increases until it reaches a ratio that stabilizes at

maturity.

The stem to root ratio is defined as: $R(t) = \frac{M_s}{M_r}$ (9)

The initial conditions where R(t=0) = 0 are known, because there is no initial stem biomass and R(t=∞) is a plateau that is reached at maturity. In this model, the logistic curve, like that of growth of the stem-to-root ratio, is assumed.

$$\frac{dR_{s\_r}}{dt} = i_{s\_r} R_{s\_r}\left[1 - \frac{R_{s\_r}}{R_{s\_r,f}}\right] \tag{10}$$

Rearranging the definition of stem-to-root ratio, $M_s = R_{s\_r}M_r$ can be substituted into equation (8)., and M_{r+s} can be re-written as :

$$M_{r+s} = M_r + RM_r = (1 + R_{s\_r})M_r \tag{11}$$

Substituting this equation into the autocatalytic equation then obtains

$$\begin{aligned}(1 + R_{s\_r})\frac{dM_r}{dt} &= i_{s\_r}(1 + R_{s\_r})M_r\left[1 - (1 + R_{s\_r})\frac{M_r}{M_{r+s,f}}\right] \\ \frac{dM_r}{dt} &= k_r M_r\left[1 - (1 + R_{s\_r})\frac{M_r}{M_{r+s,f}}\right]\end{aligned} \tag{12}$$

Summary

Cd is readily transported throughout the plant following its uptake by the roots. Assuming that the Cd desorption took place, the first step is in the intake is a metal influx across the root cell membrane, aided by transport protein *ZNT1*. Then it then has a chance of being stored in root vacuoles or transferred to the xylem for transport to the stems. Finally, the

metal influx may reach either stem or leaf cell membrane and become sequestered in their respective vacuoles. The vacuole capacity is a function of biomass, which follows the logistic growth curve.

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CHAPTER III

CASE STUDY: CADMIUM UPTAKE BY ALPINE PENNYCRESS

Introduction

The physiology and morphology of alpine pennycress make it an ideal hyperaccumulator (Assunção,*et al*., 2003). However, one attribute that may disfavor the use of alpine pennycress is its low biomass and slow growth. In order to understand how to maximize the effectiveness of phytoextraction, a mathematical model based on a solid understanding of biological mechanisms is necessary. Environmental protection agencies have used various kinds of models including empirical ones that attempt to describe observed phenomena without hypothesizing how they happen, but there are severe limitations to this approach (Collins *et al*., 2006). Alternatively, mechanistic models seek to explain how an observed phenomenon happens. One mechanistic model, developed by the University of Illinois, that simulated uptake and translocation of metals , had a simplistic basis that limits its utility for gaining deeper insights into the mechanistic processes involved (Asuncion *et al*., 2003; Sung *et al*., 2001). On the other hand, more elaborate models exist that simulate microbial interception of nutrients in the rhizosphere and age-dependent factors related to the physiology of roots, (e.g., Clarkson, 1985; Belimov *et al*. 2005). This study evaluates the ability of a physiological model of alpine pennycress, as a poor biomass accumulator but a good translocator, to capture the essence of the entire system and fundamental processes

without involving unnecessary details. Interestingly, a study done by the UK and Danish environmental agencies found that out of the six plant uptake models they examined, which ranged from simple empirical models to involved mechanistic models, the complexity of the estimation did not have any correlation with the accuracy or precision of the estimate (Collins *et al*., 2006). The purpose of this work is to develop an understanding of the magnitude of each mechanism that controls uptake, translocation and storage of Cd, from the roots all the way up to the leaves. The final model structure can hopefully be used to suggest possible management strategies for the case of Cd uptake by alpine pennycress.

Hypothesis and Model Assumptions

For the first part of the simulation, uptake performances in a hydroponic setting are compared for: (a) a hyperaccumulator alpine pennycress with a high uptake rate and a low biomass, (b) a non-accumulator field pennycress with a lower uptake rate and a similar low biomass, and (c) a much larger non-accumulator maize with a high uptake rate and a high biomass. The alpine pennycress is predicted to be an efficient tool for phytoremediation because of its faster uptake rate and higher capacity for Cd, which means that the soil can be cleaned up quicker and with fewer croppings (Macnair, 2003). Maize is expected to be a poor choice for phytoextraction because, even though the uptake rate is higher, the concentration in the biomass will still be small for a given harvest period (Das *et al*., 2007).

In the second part of the simulation experiment, plant growth is included. The hypothesis is that the toxicity limit would be reached quicker than with a mature plant due to the small initial biomass, assuming it germinates successfully. During the course of its growth, the plant would only experience a dilution effect if the uptake rate is slower than the growth rate. However, since the uptake rate is a function of biomass and since the plant is continually taking up Cd since germination, observation of the dilution effect is unlikely.

All of the plants in the model are assumed to be under idealized climatic conditions: no seasonal or diurnal variation in terms of solar radiation, water content and essential nutrient levels. Furthermore, no symbiosis with fungi or bacteria is assumed, which may enhance or inhibit further Cd uptake. The parametric values (Appendix B) used in the model are from hydroponic studies where Cd concentration is 5 μM. When plants are actually sown onto soil for real application, uptake rates of one to two orders of magnitude slower should be expected.

Input Parameters

The fundamental difference between alpine and field pennycress is the maximum uptake rate $\alpha_{r,c,max}$, the former being about six times faster than the latter (Lasat *et al.,* 1998). Other differences between alpine and field pennycresses include the sensitivity of field pennycress to the current concentration of Cd in the tissue, causing greater resistance to

uptake and translocation. The maximum concentration that the non-accumulator can handle before it reaches the phytotoxicity limit in the cytoplasm is much smaller than it is in a hyperaccumulator, yet there is a greater number of binding sites in the non-accumulator. The translocation to stem and leaf is also not observed as is the case for hyperaccumulator counterpart.

The maize has the same uptake rate assigned as the alpine pennycress, which implies the total number of sites is still high. For the purpose of this exercise, the uptake rate for maize is equivalent to that of the alpine pennycress. Therefore the only difference is the significantly larger biomass for maize in comparison to alpine pennycress.

For the growth experiment, the starting biomass is less than 1% of the mature plant used in the previous simulations and the total biomass increases according to a logistic (?) equation described in Chapter 2. The maximum uptake rate is also proportional to the ratio of the actual biomass to the maximum biomass for each time step.

Since a typical harvest interval for phytoextraction usually lies between nine to twelve weeks, the simulations were done for 1,500 hour intervals or until the plant reached the phytotoxicity limits. Also, unless explicitly stated, all the Cd concentrations in the accompanying figures are that of cytoplasm and not the vacuole/cell wall bound forms.

Results and Discussion

With phytotoxicity limits of the alpine pennycress set to 0.2, 0.9 and 1.035 mg per g for root, stem, and leaf biomass, respectively, the simulation suggests that in a hydroponic condition, the leaf concentration (green) would reach its maximum concentration after a little over 180 hours, or a week and a half (Figure 3.1). However, already 20 hours into the simulation, the root concentration (red) approaches the phytotoxicity limit and since the vacuoles are fairly fast at binding the Cd, the Cd in the bound form reaches saturation a few hours after Cd appears in each compartment (not shown).

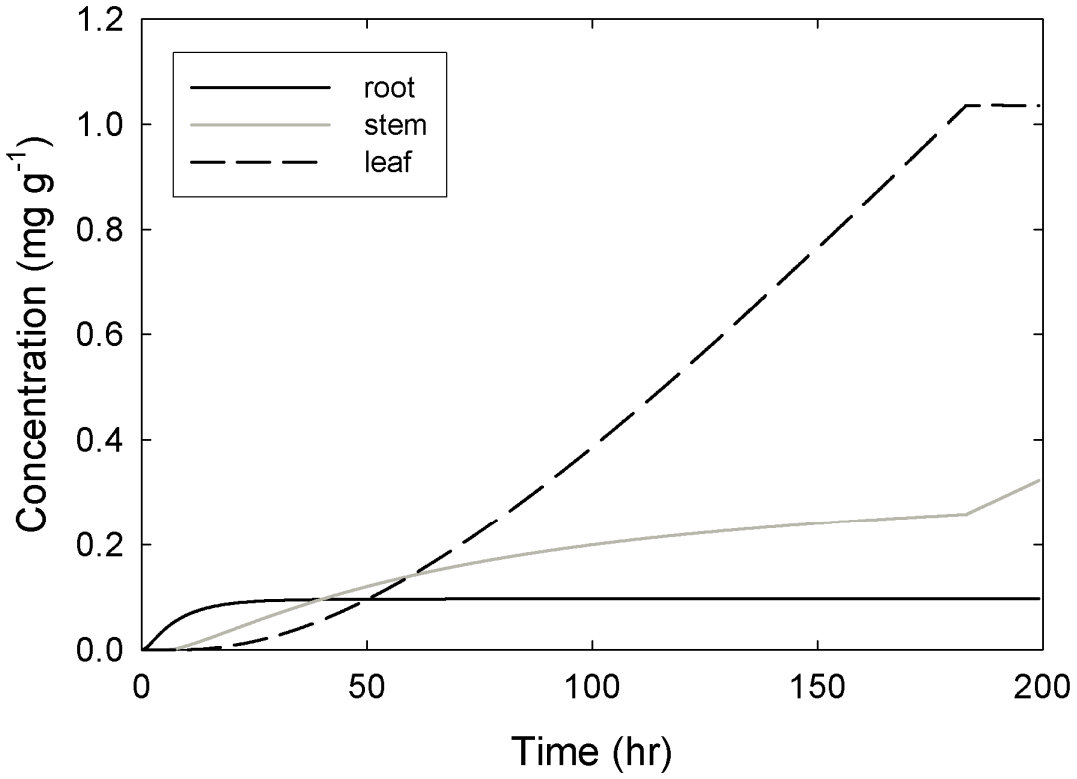

Figure 3.1: Simulation of an alpine pennycress uptake and translocation of Cd over 200 hours

A non-accumulator, unlike a hyperaccumulator, has a much lower maximum uptake rate (Figure 3.2). When only the uptake rate is varied, keeping everything else fixed, the results show a similar, but much delayed, phytotoxicity effect where the phtotoxicity limit in the leaf is reached around 1,100 hours after planting. Again, the root maximum is reached relatively early on, before 200 hours, relative to the time where the leaf maximum is reached. Because the transfer rate is high, a significant increase in the stem concentration does not take place until the leaves cannot take any more Cd.

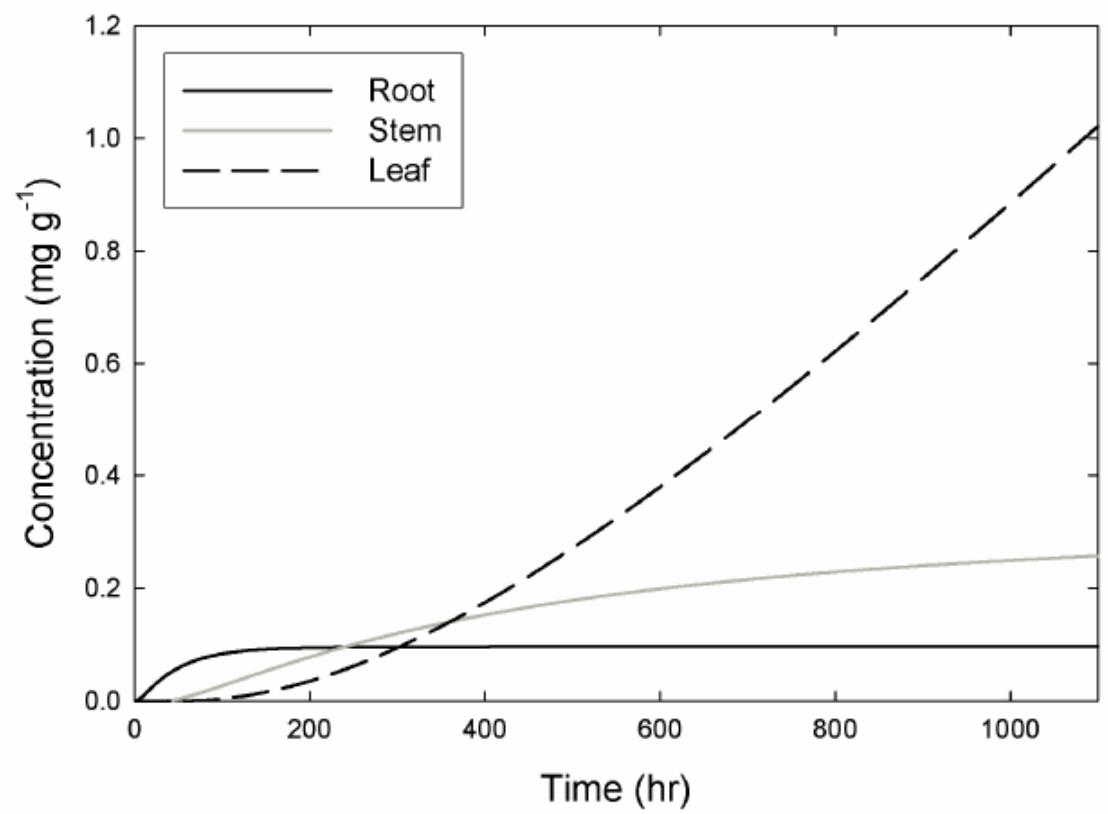

Figure 3.2: Modification of Figure 3.1 with slower uptake

When other parameters are changed to simulate field pennycress, not only did the non-accumulator take much longer time to accumulate significant Cd, but also concentrations that would be effective for harvest at the end of 1,500 hours were not reached. Field pennycress has very low absorption capacity, which is also limited by the much smaller uptake rate. For a given system, pennycress would require a much higher number of harvest cycles.

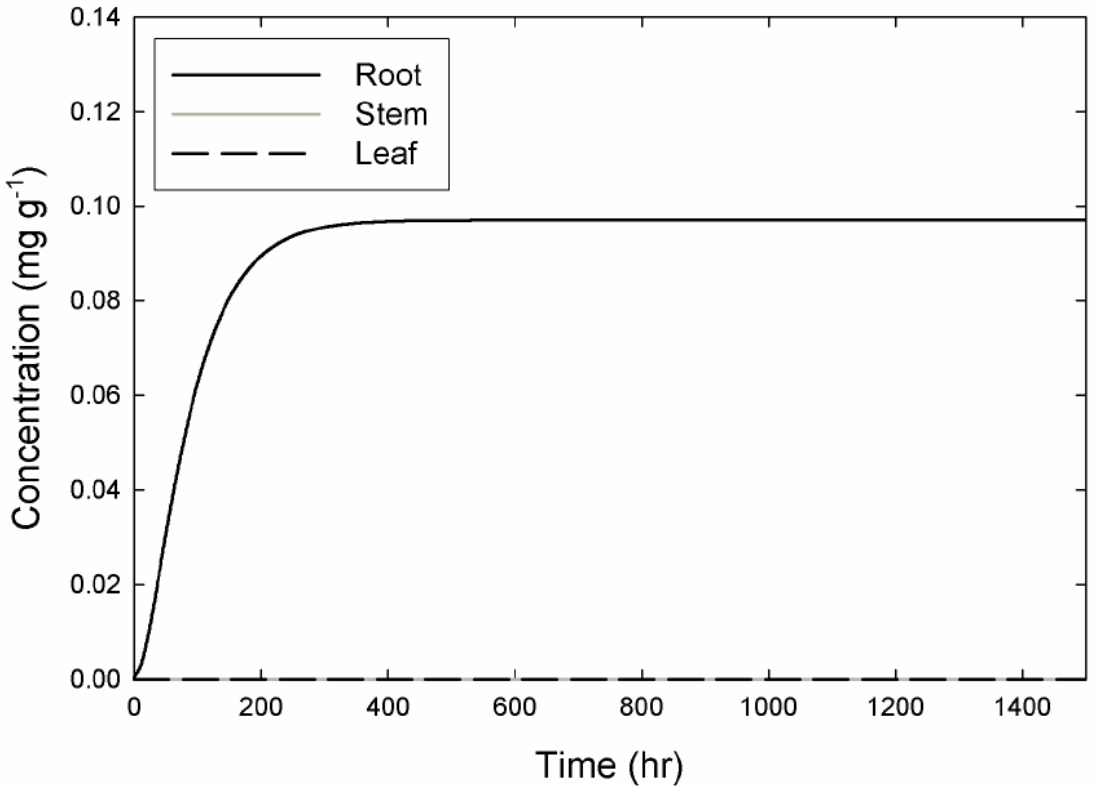

Figure 3.3: Field pennycress uptake and translocation of Cd over 1,500 hours

As expected, due to its large biomass, the maize did not yield a significant Cd concentration, even within the roots where the concentration remained 0.1 mg per g of biomass (Figure 3.4). Since the stems and leaves also have great biomass, the maize does not seem to gain a significant concentration of Cd.

As mentioned before, the maize has a large biomass but very short longevity once Cd is injected into the system. The absorbed Cd stays in the root. Extracting Cd through maize means that the metal must to be in an available and mobile form, in which a lot of the Cd may be lost from the system either through vertical or horizontal transport, usually causing more spread of the contaminant. The pore water concentration of Cd, Ni, and Zn all increase with cropping; this may seem counter-intuitive when the objective is to lower the concentration. However, note that it is the concentration in the pore water and not that of the total heavy metal concentration per mass of soil. Therefore, this may imply that, after each cropping, the roots (non-harvestable portion) release the Cd stored in that tissue and increase the total soluble Cd, at least temporarily.

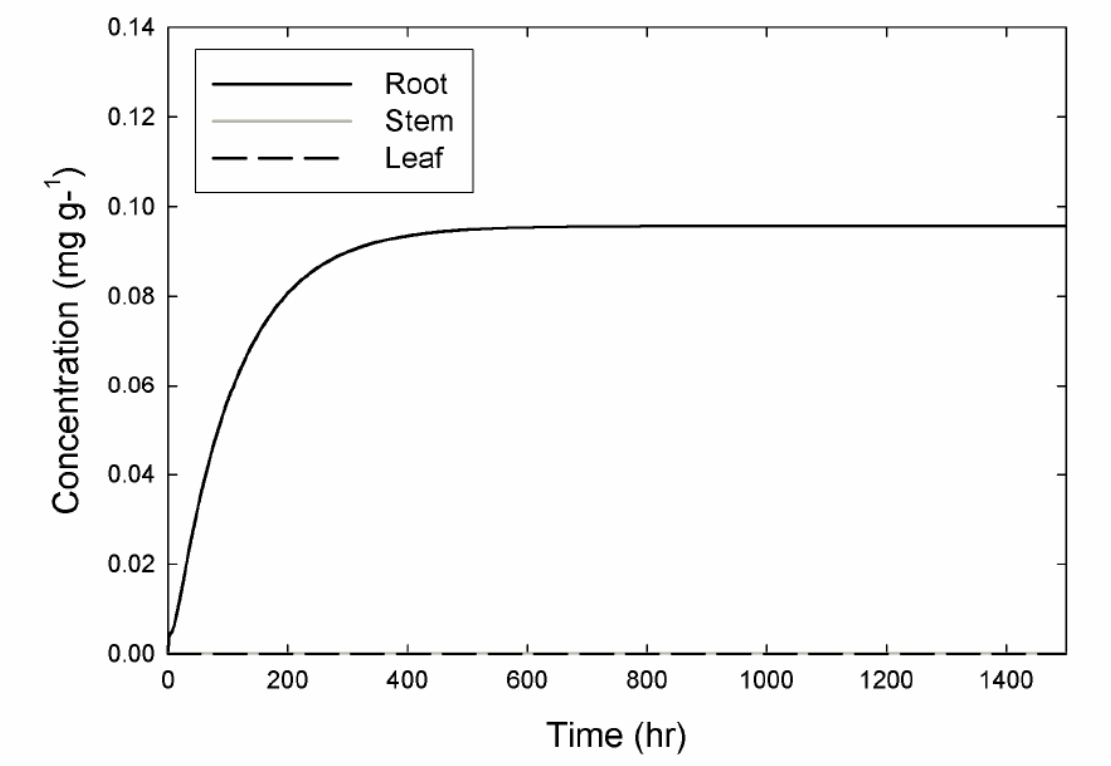

Figure 3.4: Maize uptake and translocation of Cd over 1,500 hours

For the purpose of the growth simulation exercise, in order to demonstrate a dilution effect, the hyperaccumulator was grown at an accelerated rate where the full biomass is achieved within a week, unlike the typical slow-growing pennycress (Figure 3.5). Since the growth technically has a dilution affect, the actual accumulation rate of Cd concentration in the alpine pennycress would much faster if the growth is slower than simulated (Figure 3.6).

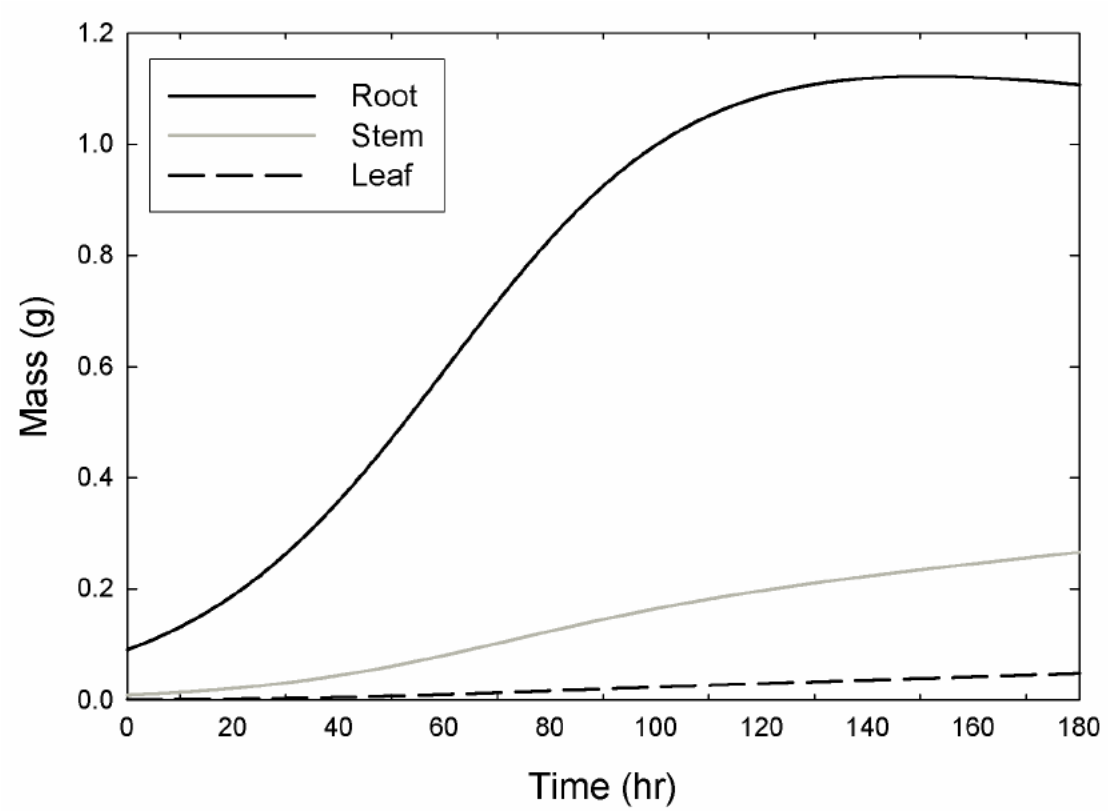

Figure 3.5: Accelerated growth curve of the alpine pennycress in the uptake and translocation simulation. The gold line is the total biomass, red for root, green for stem, blue for leaf, purple for stem to root ratio, light blue for leaf to root ratio

The growing alpine pennycress lives less than 200 hours before it is damaged by Cd (Figure 3.6). A major difference observed in this simulation is that the stem (green) concentration reaches the phytotoxicity limit first, followed quickly by the leaf (blue), as a result of the combination of fast translocation from the root and small stem biomass. Although this timing may seem similar to that of Figure 3.1, since the biomass is smaller, even if the concentration is equivalent, the total amount of Cd harvested would be much less in this case.

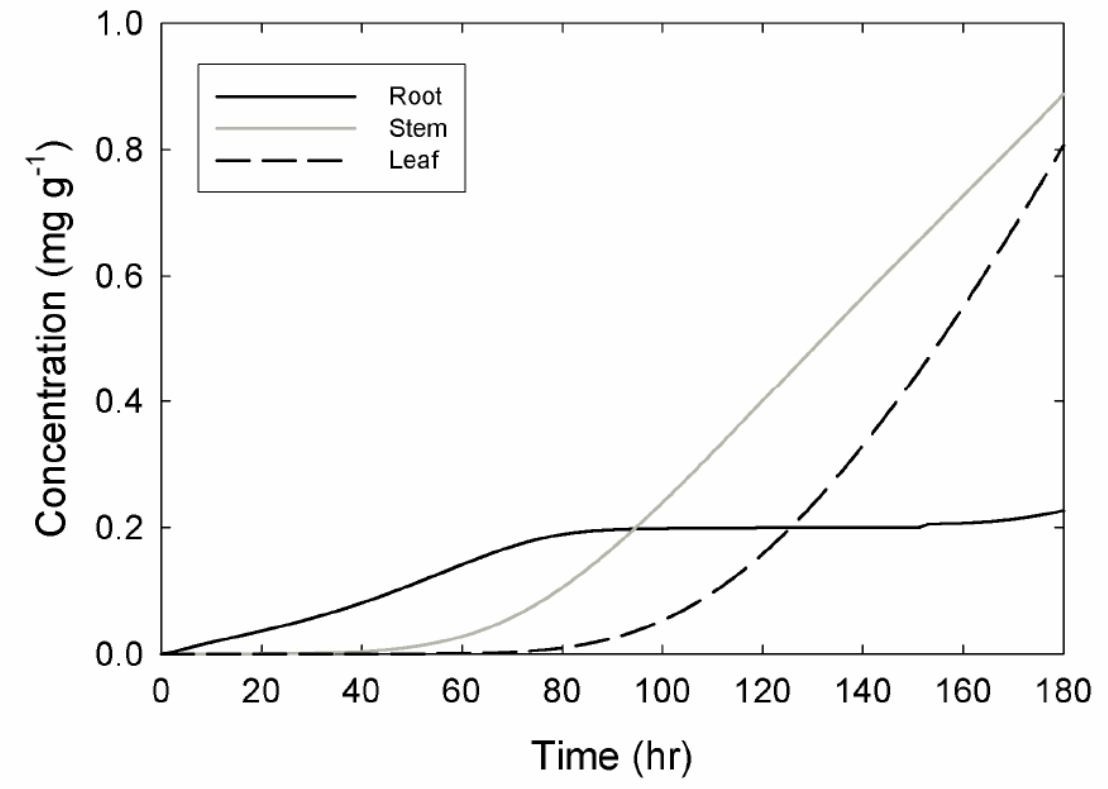

Figure 3.6: Growing alpine pennycress uptake and translocation of Cd over 180 hours

Conclusions and Recommendations for Future Work

This model is a product of both scientific understanding and mathematical application that captures the essence of the uptake, translocation and storage of a heavy metal, specifically Cd, by plants. Not only does the hyperaccumulating alpine pennycress flourish on contaminated site, but indeed prefers to situate its roots into the metal "hot spots" so that it can absorb large quantities of Cd into the roots and then transfer them into stems and leaves. The only drawback to this species is its slow growth rate and low biomass. However, even with these negative features, it still absorbs a much greater quantity of Cd than a non-accumulator with a large biomass.

Accumulation of heavy metals involves several steps, including metal transport across root cell membranes quantified by the Michaelis-Menten equation (1); xylem loading and translocation as in equation (4); and sequestration of metals in specialized leaf cells or vacuoles as in equation (2). The major conclusion from the numerical simulation is that the most important factor that affects plant contamination is the maximum uptake velocity, which governs the regional uptake by the roots.

Even though the basis of the mathematical formulae used in this model has a qualitative explanation, further data are required to calibrate and validate the model. For an example, phytotoxicity usually affects the metabolism of the plant, which is not accounted for in the model. As the concentration approaches the toxicity limit, the maximum uptake rate would also decrease due to plant's increased investment into protecting itself. Therefore the uptake rate is likely overestimated in the current model and implies that the estimated harvest time may be longer than indicated in the simulations.

Similarly, the effects of exchanges between mobile and non-exchangeable soil Cd on the uptake rate are not accounted for in this study. If the uptake rate is significantly slower than the chemical exchange, then in a moderately polluted soil, pore water Cd concentration can be assumed to stay constant until the ending stage of the clean up. On a similar note, other metals such as Zn, Fe, Cu have a competitive effect on Cd uptake (Basic *et al*. 2006; Smith and Yamanaka, 2006). Despite the lack of precise quantitative data, the results are still not

likely to change the fact that alpine pennycress still performs better than field pennycress or other species that are endemic to polluted locations.

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APPENDIX A

PECLET (Pe) NUMBER FOR TRANSPIRATION

In the Michaelis-Menten.m model, the advective transport due to transpiration is considered to be the main driving force that translocate Cd from root to stem and from stem to leaf. That is not to suggest diffusion does not occur. However, by calculating for Peclet number, a dimensionless number relating the rate of advection of a flow to its rate of diffusion,

$$Pe = \frac{LV}{D} = \frac{(10cm)(5x10^{-5} cm/s)}{10^{-6} cm^2/s} = 500 >> 1 \qquad \text{(A.1)}$$

where L is characteristic length, specifically of the plant in this case, V is velocity of transpiration and D is the diffusion coefficient of anion/cation in water, neglecting diffusion is justified.

This condition may change if the transpiration velocity is found to be slower when the temperature is lower, the humidity is higher, and/or diffusion coefficient is greater if Cd is found in more diffusive form.

APPENDIX B

PARAMETERS USED IN THE MODEL

B.1.1 PARAMETER VALUES FOR T.CAERULESCENS IN FIGURE 3.1 (UNIT, SOURCES, ACCEPTABLE RANGE, if applicable)

Symbol	Sub-category	Description	Root	Stem	Leaf
α	**c, *max***	**Maximum uptake rate into cytoplasm (work in conjunction w/ half saturation constant below)**	1.375 x 10^{-3} (mg_{Cd} g^{-1}_{root} hr^{-1}, p.61)	*Same magnitude as root*	*Same magnitude as root*
α	**b, *max***	**Maximum adsorbance rate onto cell walls/vacuoles**	Same as or slightly smaller than uptake into cytoplasm because the cell walls are very electronegative, therefore use the same as $\alpha_{[compartment],\ c,\ max}$		
β		**Surface to biomass ratio**	0.03 (cm^2/g, conservative estimate using sphere model)		
κ_O		**Half saturation constant – inter compartment**	0.45 (μM)	Assume same value as leaf	9.68 (μM, Cosio *et al*. 2004, +/- 5.78)
κ_I		**Half saturation constant – intra compartment**	Small value since V_{max} should be easily met. Approximate value of 0.1μM		
i		**Ratio increment rate**	Calculated using integration and growth rate: approx. growth rate		
k		**Growth rate**	0.1 (1/day, Trapp. 2000)	0.1 (1/day, Trapp. 2000)	0.1 (1/day, Trapp. 2000)
t	**crit**	**Critical time in which the next compartment starts growing**	15 (days, estimate from flowering timing in Bond *et al*., 2007)		N/A
			N/A	20 (days, Bond *et al*., 2007)	

B.1.2 VARIABLES VALUES FOR T.CAERULESCENS IN FIGURE 3.1 (UNIT, SOURCES, ACCEPTABLE RANGE, if applicable)

Symbol	Sub-category	Description	Root	Stem	Leaf
C	**c, *max***	**Phyto-toxicity limit (dependent on the concentration in cytoplasm)**	0.20 (mg/g, Nedelkoska and Doran, 1999)	0.920 (mg/g, Cosio et al. 2005)	1.035 (mg/g, Cosio et al. 2005)
C*	**b, *max***	**Maximum adsorbance space available on cell walls/vacuoles**	biomass x β_r	biomass x β_r	biomass x β_l
C	**c, *min***	**Minimum concentration before any net transfer of Cd into the next compartment is observed**	0 mM	0 mM	0 mM
C*	**c, *min***	**Minimum concentration necessary before any net transfer of Cd onto cell walls or into vacuoles observed**	0 mM	0 mM	0 mM
B	***final***	**Final biomass (in optimal condition)**	0.2 (g for root, Roosens *et al*., 2003, *conservative*) or 1.43 g for the entire plant		
R	***max***	**Ratio increment rate (in optimal condition)**	1.62 = stem::root (unitless, DeChamps *et al*., 2005, *conservative; up to 1.70*)		
				0.43 = leaf::stem (unitless, G.S. Banuelos et al. 2002, 0.38 to 0.44)	

APPENDIX C

MODEL ALGORITHM

The MATLAB code below is for growth simulation specifically, however, the format from one to another is identical and the only thing that differ from one case study to another is the parameter values.

```
%ACTIVE Transport to roots and passively to stems and leaves
% Hope to demonstrate the highest (though realistic) uptake by
% T.caerulescens - hyperaccumulator WITH GROWTH and no harvest



%--------------------------------------------------------------------------
% ----------------------------USER INPUT ----------------------------------
% --------------------------------------------------------------------------

% TIME
% BIOMASS OF THE PLANT
   Mass.final = 1.43; % [g] Total biomass of a mature plant (including leaf)
   Mass.start = 0.1; % [g] Starting seed biomass

% PLANT GEOMETRY RATIO IN OPTIMAL CONDITION[IF SPECIFIED]
 Ratio.stem_root.mature = 1.62; % Final stem to root biomass ratio @ maturity
 Ratio.stem_root.start = 0.1; % (Optional) Non-zero starting ratio for stem to root @ t = critical stem growth + dt
 Ratio.leaf_stem.mature = 0.43; % Final leaf to stem ratio @ maturity (G.S. BANUELOS ET AL. 2002)
 Ratio.leaf_stem.start = 0.1; % (Optional)Non-zero starting ratio for leaf area to total weight @ t = critical stem growth + dt

% CRITICAL TIME UNDER IDEAL CONDITIONS: 20 DEGREE CELSIUS, PLENTY OF WATER, ENOUGH
SUNLIGHT...ETC
 Time.crit.stem = 0; % [hrs] Amount of time it takes to grow stem
 Time.crit.leaf = 0; % [hrs] Amount of time it takes to grow leaf

%      ----- NOT USED MATERIAL -------------------
%INCLEMENT WEATHER CONDITION

%DECLARATION/DEFINITION OF VARIABLES
%--------------------------------------------------------------------------
%----------------------------------TIME----------------------------------
%--------------------------------------------------------------------------
```

```
% Time scales and steps
tmin = 1; % Starting time
tmax = 200; % [total hours-tmin] length of the experiment
tnow = tmin; % counter for the current time
dt = 0.01; % [hr] time step

tstepmax = (tmax-tmin)/dt + tmin; % max number of steps for the for-loop
tstep = tmin:dt:tmax;

t.lag.root_stem = 0; % [hr] the traveling time from active transport site to xylem and into stem cell
t.lag.root_leaf = 0; % [hr] the traveling time from active transport site to xyltem and into leaf cell
% ---------------------------------------------------------------------------
% ----------------------------CONCENTRATION------------------------------
% ---------------------------------------------------------------------------
%[mg/kg for biomass concentration and micro-mol for solution]
% current concentration
c.sol.cur = 5; % [micro-M] Current solution concentration @ root surface with initial value
c.lroot.cur = 0; % Current root concentration in liquid form with initial value
c.lstem.cur = 0; % Current stem concentration in liquid form with initial value
c.lleaf.cur = 0; % Current leaf concentration in liquid form with initial value
c.sroot.cur = 0; % Current root concentration in solid form with initial value
c.sstem.cur = 0; % Current stem concentration in solid form with initial value
c.sleaf.cur = 0; % Current leaf concentration in solid form with initial value

% one-step future concentration
c.sol.new = 0; % New solution concentration @ root surface with initial value
c.lroot.new = 0; % New root concentration in liquid form with initial value
c.lstem.new = 0; % New stem concentration in liquid form with initial value
c.lleaf.new = 0; % New leaf concentration in liquid form with initial value
c.sroot.new = 0; % New root concentration in solid form with initial value
c.sstem.new = 0; % New stem concentration in solid form with initial value
c.sleaf.new = 0; % New leaf concentration in solid form with initial value

% Constant concentrations; characteristics inherent to the plants
% Phytotoxicity limit (in cytoplasm + cell walls/vacuoles)
c.root.max = 0.20;% [mg/g] concentration at which the metal kills the root
c.stem.max = 0.90;% [mg/g] concentration at which the metal kills the stem
c.leaf.max = 1.035;%[mg/g] concentration at which the metal kills the leaf
```

```
% Minimum concentration before flow
c.sol.min = 0; % [micro-M]concentration at which, if below, there is no net flow from soil to root
c.root.min = 0; % [micro-M] concentration at which, if below, there is no net flow from root to stem
c.stem.min = 0; % [micro-M] concentration at which, if below, there is no net flow from stem to leaf

% Binding site capcity; may vary with surface area growth ** TO BE
% CALCULTED LATER - ONLY DECLARING AT THIS STAGE
c.root.cap = 0;% [mg/g]concentration of the root that is bound to cell wall/vacuoles
c.stem.cap = 0;% [mg/g]concentration of the stem that is bound to cell wall/vacuoles
c.leaf.cap = 0;% [mg/g]concentration of the leaf that is bound to cell wall/vacuoles

% capacity of Cd per area [mg/g cm^2] - depending on adsorption type
capacity.area.root = 1;
capacity.area.stem = 1;
capacity.area.leaf = 1;

% Concentration matrix
c.system = zeros(3, tstepmax); % concentration made in matrix form for each time step
c.lsystem = zeros(3, tstepmax); % concentration of each compartment in liquid form for each time step
c.ssystem = zeros(3, tstepmax); % concentration of each compartment in solid form for each time step



%-----------------------------------------------------------------------
%-------------------------------MASS-------------------------------------
%-----------------------------------------------------------------------

% ---------------PROCESSING OF USER-DEFINED INPUT-------------------------
% Ratio of leaf to total
Ratio.leaf_root.mature = Ratio.stem_root.mature * Ratio.leaf_stem.mature;

% maturity stem +root biomass is final total mass minus final leaf mass
Mass.stem_root.final = Mass.final-Ratio.leaf_root.mature/(Ratio.leaf_stem.mature + Ratio.leaf_root.mature +
Ratio.leaf_root.mature);

% Ratio of leaf to total
Ratio.leaf_root.mature = Ratio.stem_root.mature * Ratio.leaf_stem.mature;

% Fraction difference betwee starting biomass and total biomass @ maturity
f.mass.total = (Mass.final-Mass.start)/Mass.start;
```

```
f.mass.stem_root = 0.9999;
f.mass.leaf_stem = 0.9999;

% Fraction different between starting ratio and "mature" ratio
f.ratio.stem_root = (Ratio.stem_root.mature - Ratio.stem_root.start)/Ratio.stem_root.start;
f.ratio.leaf_stem = (Ratio.leaf_stem.mature - Ratio.leaf_stem.start)/Ratio.leaf_stem.start;

% Growth increment parameters
% 99.99% maturity weight and ratio is understood as a full grown plant
% i.e. product.log.mass =
% log((Mass.final-0.9999*Mass.final)/0.9999*Mass.final)-log((Mass.final-Mass.start)/(Mass.start))
product.log.mass.total = reallog(0.0001)- reallog(f.mass.total);
product.log.mass.stem_root = reallog(0.0001)- reallog(f.mass.stem_root);
product.log.mass.leaf_stem = reallog(0.0001) - reallog(f.mass.leaf_stem);
product.log.ratio.stem_root = reallog(0.0001)-reallog(f.ratio.stem_root);
product.log.ratio.leaf_stem = reallog(0.0001)-reallog(f.ratio.leaf_stem);

%-----------------------------BIOMASS-------------------------------------
% Biomass at present - relates to the carrying capacity of the compartment
m.root.cur = Mass.start/(Ratio.stem_root.start + Ratio.stem_root.start*Ratio.leaf_stem.start+1);    % [g]
m.stem.cur = m.root.cur*Ratio.stem_root.start;    % [g]
m.leaf.cur = m.stem.cur*Ratio.leaf_stem.start;    % [g]
m.total.cur = Mass.start; % [g]

m.stem_root.crit = 0; % Mass of stem and root at t.crit.leaf

m.stem_root.cur = m.root.cur + m.stem.cur;

% Biomass at one-step in the future - relates to the carrying capacity of
% the compartment
m.root.new = m.root.cur;
m.stem.new = m.stem.cur;
m.leaf.new = m.leaf.cur;
m.total.new = m.total.cur;

% Biomass growth rate [1/hr]
k.growth.root = 0.004167;
k.growth.stem = k.growth.root;
k.growth.leaf = k.growth.root;
```

```
% Maximum biomass [g]
 m.root.max = Mass.final/(Ratio.leaf_root.mature + Ratio.stem_root.mature + 1);
 m.leaf.max = Ratio.leaf_root.mature*m.root.max;
 m.stem_root.max = Mass.final-m.leaf.max;
 % maturity stem +root biomass is final total mass minus final leaf mass
 %m.root.max = m.stem_root.max/(1+Ratio.stem_root.mature);
 m.total.max = Mass.final;

% Biomass ratio at present
 r.stem_root.cur = Ratio.stem_root.start;
 r.leaf_stem.cur = Ratio.leaf_stem.start;

% Biomass ratio in the future
 r.stem_root.new = r.stem_root.cur;
 r.leaf_stem.new = r.leaf_stem.cur;

% Maximum biomass ratio
r.stem_root.max = Ratio.stem_root.mature;
 r.leaf_stem.max = Ratio.leaf_stem.mature;

% Critical time for stem and leaf growth (initiate ratio growth)
 t.crit.stem = tmin + Time.crit.stem; % [hr] time where critical mass for root is reached
 t.crit.leaf = tmin + Time.crit.leaf; % [hr] time where critical mass for stem is reached

% Ratio growth rate
% Note: Ratio reaches until 99.99 % of the full biomass and ratio is
% approximated as a full grown plant
 k.ratio.stem_root = product.log.ratio.stem_root/(product.log.mass.stem_root/k.growth.root-t.crit.stem*dt);
 k.ratio.leaf_stem = product.log.ratio.leaf_stem/(product.log.mass.leaf_stem/k.growth.leaf-t.crit.leaf*dt);

% mass matrix
m.system = zeros(3, tstepmax); % biomass over time
m.lsystem = zeros(3, tstepmax);% Cd in cytoplasm over time
m.ssystem = zeros(3, tstepmax);% Cd bound in cell walls/vacuoles over time

% ratio matrix
%r.system = zeros (2, tstepmax); % stem:root ratio over time
```

```
% mass + ratio matrix
m_r.system = zeros (6, tstepmax);

%----------------------MASS OF CADMIUM-----------------------------------
% Mass of total Cd in each compartment at present
m.lroot.cur = 0; % Current root concentration in liquid form with initial value
m.lstem.cur = 0; % Current stem concentration in liquid form with initial value
m.lleaf.cur = 0; % Current leaf concentration in liquid form with initial value
% Surface area of total Cd in each compartment at present
m.sroot.cur = 0; % Current root concentration in solid form with initial value
m.sstem.cur = 0; % Current stem concentration in solid form with initial value
m.sleaf.cur = 0; % Current leaf concentration in solid form with initial value

% Mass of total Cd in each compartment at one-step in the future
m.lroot.new = 0; % New root concentration in liquid form with initial value
m.lstem.new = 0; % New stem concentration in liquid form with initial value
m.lleaf.new = 0; % New leaf concentration in liquid form with initial value
% Surface area of total Cd in each compartment at one-step future
m.sroot.new = 0; % New root concentration in solid form with initial value
m.sstem.new = 0; % New stem concentration in solid form with initial value
m.sleaf.new = 0; % New leaf concentration in solid form with initial value

%-------------------------------------------------------------------------
%--------------------------UPTAKE RATE-----------------------------------
%-------------------------------------------------------------------------

%transfer rate [initially set to unity]
    % transfer from one compartment to another (i.e. solution to root)
        % max rate for the given condition (age, weather)
        a.root.max = 0.000013752*(m.root.cur/m.root.max); % [g/g hr]fastest transfer rate possible to root [nmol/(g*min),
0.0397 nmol/(g*sec)]
        a.stem.max = a.root.max* (m.leaf.cur/m.leaf.max); % [mg/g hr]fastest transfer rate possible to stem
        a.leaf.max = a.root.max * (m.leaf.cur/m.leaf.max); % [mg/g hr]fastest transfer rate possible to leaf
        % actual transfer rate to next compartment ** TO BE CALCULTED LATER
        a.lroot = 0; % actual transfer rate to root
        a.lstem = 0; % actual transfer rate to stem
        a.lleaf = 0; % actual transfer rate to leaf

    % transfer within a compartment (i.e. to/from cell walls/vacuoles and
```

```
    % liquid form).    This parameter indicates how fast the Cd compounds can
    % bind to the solid structure
        % max rate for given condition
        a.sub.root.max = 0.9 * a.root.max; % [g/g hr] transfer rate for the root metal to partition to cell wall/vacuoles
        a.sub.stem.max = 0.9 * a.stem.max; % [g/g hr] transfer rate for the stem metal to partition to cell wall/vacuoles
        a.sub.leaf.max = 0.9 * a.leaf.max; % [g/g hr] transfer rate for the leaf metal to partition to cell wall/vacuoles
        % actual transfer rate to next compartment (this may be negative
        % value)
        a.sroot = 1; % actual transfer rate from liquid to cell walls/vacuoles in root
        a.sstem = 1; % actual transfer rate from liquid to cell walls/vacuoles in stem
        a.sleaf = 1; % actual transfer rate from liquid to cell walls/vacuoles in leaf

    a.lsystem= zeros(3,tstepmax); % Keep track of the uptake rates in cytoplasm
    a.ssystem= zeros(3,tstepmax); % Keep track of the uptake rates in cytoplasm
%-------------------------------------------------------------------------
%-------------------------------OTHERS-----------------------------------
%-------------------------------------------------------------------------
% half saturation    [micro-M]
k.soil = 0.45; %Michaelis-Menten constant, aka half saturation constant
k.stem = 9.68; % describes the difficulty transporting from root to stem
k.leaf = 9.68; % describes the difficulty transporting from stem to leaf
k.sub.root = 0.0000000000001; % permeability constant for root vacuoles
k.sub.stem = 0.0000000000001; % permeability constant for stem vacuoles
k.sub.leaf = 0.0000000000001; % permeability constant for leaf vacuoles
c.total = zeros(3, tstepmax); % total concentration

% Interior surface-biomass equivalent ratio
beta.root = 0.03; % [cm^2/g] root ratio, which is expected to be greater value due to its thin nature
beta.stem = 0.03; % [cm^2/g] stem ratio
beta.leaf = 0.03; % [cm^2/g] leaf ratio, which is expected to be greater value due to its flat nature

% Plot and movie parameters.
% v = 0; %Movie frame counter
% han = plot (x,cold); % Handle for plotting
% set (han, 'LineWidth', 2) % Sets plot parameters
% axis([xmin xmax 0 100]) % Modify concentration as the intial cond.
% nframes = 100; % Number of frames
% temp1 = tmax/nframes; % Conditional for taking frames
```

```
%-------------------------------------------------------------------
%-------------------------DATA PROCESSING-----------------------------
%-------------------------------------------------------------------

% Initial condition
v = 0;
for(tnow = tmin:tstepmax) % repeats for tmax amount of time
    %-------------------------------------------------------------------
    %----------------GROWTH OF THE PLANT --------------------------------
    %-------------------------------------------------------------------

    % Solving for the ratio
    % Stem to root ratio using logistic curve
     if (tnow>=t.crit.stem)
     r.stem_root.new = r.stem_root.cur + k.ratio.stem_root * r.stem_root.cur *(1-(r.stem_root.cur/r.stem_root.max))* dt;
     end
     if(tnow == t.crit.stem)
     m.stem.cur = r.stem_root.cur*m.root.cur;
     end
    % Leaf area to stem biomass ratio using logistic curve as long as stem
    % has a biomass and new biomass calculation
     if(tnow>=t.crit.leaf)
     r.leaf_stem.new = r.leaf_stem.cur + k.ratio.leaf_stem * r.leaf_stem.cur * (1-(r.leaf_stem.cur/r.leaf_stem.max))* dt;
     if(tnow == t.crit.leaf)
         m.leaf.cur = r.leaf_stem.cur*m.stem.cur;
     end
     m.total.cur = m.root.cur + m.stem.cur + m.leaf.cur; % Total mass calculation
     m.total.new = m.total.cur + 0.5 * k.growth.leaf * m.total.cur*(1-(m.total.cur/m.total.max));
     end

    m.root.new = m.total.new/(r.leaf_stem.new * r.stem_root.new + r.stem_root.new + 1);
     if (tnow>=t.crit.stem)
     m.stem.new = r.stem_root.new* m.root.new;
     end
    % Leaf biomass update which is solved by knowing the ratio between the
    % leaf surface area and the total mass
    if (tnow>=t.crit.leaf)
     m.leaf.new = r.leaf_stem.new * m.stem.new;
    end
```

```
 % Root + stem biomass update
 %if(m.root.cur <= m.root.max)% only if the
% maximum root biomass isn't reached
 %m.stem_root.new = m.stem_root.cur + k.growth.root* (1-(m.stem_root.cur/m.stem_root.max))*dt;
% Root biomass update
% m.root.new = m.root.cur + k.growth.root* m.root.cur * (1-(m.root.cur/m.root.max))*dt;
 %m.root.new = m.stem_root.new/(1+r.stem_root.new);
%end
% Stem biomass update which is solved by knowing the ratio between stem
% and root
% if (tnow>=t.crit.stem)
% m.stem.new = r.stem_root.new* m.root.new;
% end
% Leaf biomass update which is solved by knowing the ratio between the
% leaf surface area and the total mass
%    m.leaf.new = r.leaf_stem.new * m.stem.new;

m.system(3,tnow) = m.root.cur; %Storing root biomass information for current time step
m.system(2,tnow) = m.stem.cur; %Storing stem biomass information for current time step
m.system(1,tnow) = m.leaf.cur; %Storing leaf biomass information for current time step

 m_r.system(6, tnow) = m.total.cur;
 m_r.system(5, tnow) = r.stem_root.cur;
 m_r.system(4, tnow) = r.leaf_stem.cur;
 m_r.system(3,tnow) = m.root.cur; %Storing root biomass information for current time step
 m_r.system(2,tnow) = m.stem.cur; %Storing stem biomass information for current time step
 m_r.system(1,tnow) = m.leaf.cur; %Storing leaf biomass information for current time step

%-----------------------------------------------------------------------
%------RE-CALCULATING THE MAX ADSORPTION AND UPTAKE RATE---------------
%-----------------------------------------------------------------------


a.root.max = 0.000013752*(m.root.cur/m.root.max); % [g/g hr]fastest transfer rate possible to root
a.stem.max = a.root.max * (m.leaf.cur/m.leaf.max); % [mg/g hr]fastest transfer rate possible to stem
a.leaf.max = a.root.max * (m.leaf.cur/m.leaf.max); % [mg/g hr]fastest transfer rate possible to leaf

 % max concentration of the root that is bound to cell wall/vacuoles
```

```
c.root.cap = beta.root * m.root.cur* capacity.area.root;
% max concentration of the stem that is bound to cell wall/vacuoles
c.stem.cap = beta.stem * m.stem.cur * capacity.area.stem;
% max concentration of the leaf that is bound to cell wall/vacuoles
c.leaf.cap = beta.leaf * m.leaf.cur * capacity.area.leaf;

%---------------------------------------------------------------------
%----------------TRANSPORT FROM SOIL TO ROOT--------------------------
%---------------------------------------------------------------------
% NOTE: Gain/loss is only from the liquid standpoint

% local root concentration tracker
c.lroot.postLocal = 0;
m.lroot.postLocal = 0;
cond.stem = 1; % possibility of calculating transfer to stem (1 or 0, if none)

% SOIL ACTIVE TRANSPORT
% regional gain: transfer rate from solution to root
if (c.sol.cur>c.sol.min)
% checks if there is enough soil solution concentration to transfer into the
% The maximum tranfer capacity for specific solution concentration was defined above
% actual tranfer rate is calculated using Michaelis-Menton formula
a.lroot = a.root.max *((c.sol.cur-c.sol.min)/(k.soil +
(c.sol.cur-c.sol.min)))^(1)*(1-(c.lroot.cur/c.root.max))^(m.root.max/m.root.cur);
% 'a.lroot' describes the change of concentration with respect time.
% 'a.root.max' is the fastest transport rate from the soil solution
% into the root.    Michaelis-Menten type of uptake is also regulated by
% the proximity to phytotoxicity limit
else
    a.lroot = 0;
end

% local loss: transfer rate root in liquid form to root in solid form
% Transfer to the solid-bound phase is a function of the concentration
% in cytoplasm and the how close the solid-bound phase is to reaching the maximum capacity
    a.sroot = a.sub.root.max * (c.lroot.cur/(k.sub.root + c.lroot.cur))^(1)*(1-(c.sroot.cur/c.root.cap))^(1);

% --------calculatting he cytoplasm and cell wall-bound concentration after local "loss" ----
```

```
            m.lroot.cur = c.lroot.cur/1000 * m.root.cur; % [g/g]
            m.sroot.cur = c.sroot.cur/1000 * m.root.cur; % [g/g]
            % Express curr ent Cd content in terms of mass; converted from mg/g
            % to g/g

            a.diff.root1 = a.lroot - a.sroot;
            % local variable that shows the difference between the regional gain and local loss
            if (a.diff.root1 > 0)
            m.lroot.postLocal = m.lroot.cur + ((m.root.cur + m.root.new)/2) *((a.diff.root1)* dt);
            m.sroot.new = m.sroot.cur + ((m.root.cur + m.root.new)/2)*(a.sroot*dt);
            % The new Cd in cytoplasm = current + regional gain - local loss
            % The new Cd in cell wall-bound = current + local "loss"
            else
            m.sroot.new = m.sroot.cur + ((m.root.cur + m.root.new)/2)*(a.lroot*dt);
            m.lroot.postLocal = m.lroot.cur;
            % The new Cd in cytoplasm = current; NO gain because everything is
            % gone to the bound form
            % The new Cd in cell wall-bound = current + what was available from
            % regional gain
            cond.stem = 0;
            end

            c.lroot.postLocal = m.lroot.postLocal / m.root.new * 1000; %[mg/g]
            % The new Cd bound is due to local "gain"
            c.sroot.new = m.sroot.new/m.root.new * 1000; % [mg/g]
            % Converting the Cd content back in terms of concentration which
            % may or may not the dilute the previous concentration depending on
            % the growth

        %---------------------------------------------------------------------
            % regional loss: transfer rate from root to stem
        t.lag.diff = tnow - t.lag.root_stem/dt-1;
        if (tnow > tmin && cond.stem == 1 && t.lag.diff > 0 && c.lsystem(3, t.lag.diff) >= c.root.min && m.stem.cur > 0 &&
c.lstem.cur < c.stem.max)
            % Insure that there's no back flow from stem to root by checking...
            % enough lag time is present
            % there is enough concentration to result in net positive flow to
            % stem; stem actually has biomass; and stem concentration is NOT
            % saturated
```

```
a.lstem = a.stem.max*((c.lsystem(3, (t.lag.diff))-c.root.min)/(c.root.max - c.root.min ))^(1)*(1-(c.lstem.cur/(k.stem +
c.stem.max)))^(m.leaf.max/m.leaf.cur);
check = 100;
% a.k.a. stem's regional gain
else
    a.lstem = 0; % if the threshold is not met, no transfer of Cd
    check = 2;
end



% calculatting he next time step concentration
    % update liquid concentration: new = temporary current - regional loss
    a.diff.root2 = a.diff.root1 - a.lstem;
    if (a.diff.root2 > 0)
        % if the demand from the stem is smaller than that of the supply
        % from the root
        m.lroot.new = m.lroot.postLocal + ((m.root.cur + m.root.new)/2) *(- a.lstem* dt);
        % the demanded amount is subtracted from the root
        check2 = 100;
    else
        % if the demand from the stem is larger than that of the supply
        % from the root
        m.lroot.new = m.lroot.postLocal + ((m.root.cur + m.root.new)/2) *(- a.diff.root2* dt);
        % all the can supplied from the root is subratected from the
        % root
        check2 = 2;
    end
    % The new Cd in cytoplasm = regional gain - local loss - regional loss.
    % Average of the two time step (current & future) are taken.
    c.lroot.new = m.lroot.new/m.root.new * 1000; %[mg/g]
    % Converting the Cd content back in terms of concentration which
    % may or may not the dilute the previous concentration depending on
    % the growth



% store concentration information for the time step
c.system(3,tnow) = c.lroot.cur + c.sroot.cur; % concentration total for the compartment for current time step
c.lsystem(3,tnow) = c.lroot.cur; % concentration of each compartment in liquid form for current time step
```

```
c.ssystem(3,tnow) = c.sroot.cur; % concentration of each compartment in solid form for current time step
m.lsystem(3,tnow) = m.lroot.cur; % mass of Cd floating in cytoplasm for current time step
m.ssystem(3,tnow) = m.sroot.cur; % mass of Cd bound to the cell walls/vacuoles for current time step
a.lsystem(3,tnow) = a.lroot;
a.ssystem(3,tnow) = a.sroot;
%-----------------------------------------------------------------------
%-----------------TRANSPORT FROM ROOT TO STEM -------------------------
%-----------------------------------------------------------------------

if (cond.stem == 1)% No transfer will occur as long as there is no biomass in the stem
% regional gain: transfer rate from root to stem
% ALREADY BEEN CALCULATED ABOVE AS "a.lstem"

% local root concentration tracker
c.lstem.postLocal = 0;
m.lstem.postLocal = 0;
cond.leaf = 1; % possibility of calculating transfer to stem (1 or 0, if none)

% local loss: transfer rate root in liquid form to root in solid form
% Process that transfers from the solid-bound phase to cytoplasm and
% vice versa is identical to that of the root
    a.sstem = a.sub.stem.max * (c.lstem.cur/(k.sub.stem + c.lstem.cur))^(1)*(1-(c.sstem.cur/c.stem.cap))^(1);

 % --------calculatting he cytoplasm and cell wall-bound concentration after local "loss" ----

    m.lstem.cur = c.lstem.cur/1000 * m.stem.cur; % [g/g]
    m.sstem.cur = c.sstem.cur/1000 * m.stem.cur; % [g/g]
    % Express current Cd content in terms of mass; converted from mg/g
    % to g/g

    aa.diff.stem1 =((m.root.cur + m.root.new)/2) * a.lstem - ((m.stem.cur + m.stem.new)/2) * a.sstem;
    aa.diff.stem.rev1 = ((m.stem.cur + m.stem.new)/2) * a.sstem - ((m.root.cur + m.root.new)/2) * a.lstem;

    % local variable that shows the difference between the regional gain and local loss
    if (aa.diff.stem1 > 0)
    m.lstem.postLocal = m.lstem.cur + (aa.diff.stem1 * dt);
    % mass of ROOT is correct for conservation of mass
    m.sstem.new = m.sstem.cur + ((m.stem.cur + m.stem.new)/2)*(a.sstem * dt);
    % The new Cd in cytoplasm = current + regional gain - local loss
```

```
            % The new Cd in cell wall-bound = current + local "loss"
            else
            m.lstem.postLocal = m.lstem.cur;
            m.sstem.new = m.sstem.cur + (aa.diff.stem.rev1 * dt);
            % The new Cd in cytoplasm = current; NO gain because everything is
            % gone to the bound form
            % The new Cd in cell wall-bound = current + what was available from
            % regional gain
            cond.leaf = 0;
            end

            c.lstem.postLocal = m.lstem.postLocal / m.stem.new * 1000; %[mg/g]
            % The new Cd bound is due to local "gain"
            c.sstem.new = m.sstem.new/m.stem.new * 1000; % [mg/g]
            % Converting the Cd content back in terms of concentration which
            % may or may not the dilute the previous concentration depending on
            % the growth

        %-------------------------------------------------------------------------
            % regional loss: transfer rate from root to stem
        t.lag.diff2 = tnow - t.lag.root_leaf/dt-1;
        if (tnow > tmin && cond.leaf == 1 && t.lag.diff2 > 0 && c.lsystem(2, t.lag.diff2) > c.stem.min && m.leaf.cur > 0 &&
c.lleaf.cur < c.leaf.max)
            % Insure that there's no back flow from stem to root by checking...
            % enough lag time is present
            % there is enough concentration to result in net positive flow to
            % leaf; leaf actually has biomass; and leaf concentration is NOT
            % saturated
        a.lleaf = a.leaf.max*((c.lsystem(2, (t.lag.diff2))-c.stem.min)/(c.stem.max - c.stem.min))^(1)*(1-(c.lleaf.cur/(k.leaf +
c.leaf.max)))^(m.leaf.max/m.leaf.cur);
        % a.k.a. stem's regional gain
        else
            a.lleaf = 0; % if the threshold is not met, no transfer of Cd
        end



        % calculatting he next time step concentration
            % update liquid concentration: new = temporary current - regional loss
            aa.diff.stem2 = aa.diff.stem1 - a.lleaf * ((m.stem.cur + m.stem.new)/2);
```

```
    if (aa.diff.stem2 > 0)
        cond.leaf = 1;
        m.lstem.new = m.lstem.postLocal + ((m.stem.cur + m.stem.new)/2) *(- a.lleaf* dt);
    else
        cond.leaf = 0;
        m.lstem.new = m.lstem.postLocal;
        a.lleaf = aa.diff.stem1/((m.stem.cur + m.stem.new)/2);
    end
    % The new Cd in cytoplasm = regional gain - local loss - regional loss.
    % Average of the two time step (current & future) are taken.
    c.lstem.new = m.lstem.new/m.stem.new * 1000; %[mg/g]
    % Converting the Cd content back in terms of concentration which
    % may or may not the dilute the previous concentration depending on
    % the growth

% store concentration information for the time step
c.system(2,tnow) = c.lstem.cur + c.sstem.cur; % concentration total for the compartment for current time step
c.lsystem(2,tnow) = c.lstem.cur; % concentration of each compartment in liquid form for current time step
c.ssystem(2,tnow) = c.sstem.cur; % concentration of each compartment in solid form for current time step
m.lsystem(2,tnow) = m.lstem.cur; % mass of Cd floating in cytoplasm for current time step
m.ssystem(2,tnow) = m.sstem.cur; % mass of Cd bound to the cell walls/vacuoles for current time step
a.lsystem(2,tnow) = a.lstem;
a.ssystem(2,tnow) = a.sstem;
%-----------------------------------------------------------------------
%-----------------TRANSPORT FROM STEM TO LEAF -------------------------
%-----------------------------------------------------------------------

if (cond.leaf == 1) % No transfer to leaf as long as there is no biomass
    % and less than half of the binding sites in the stem is filled
% regional gain: transfer rate from stem to leaf
% ALREADY BEEN CALCULATED ABOVE AS "a.lleaf"

% local loss: transfer rate root in liquid form to root in solid form
% Transfer to the solid-bound phase is a function of the concentration
% in cytoplasm and the how close the solid-bound phase is to reaching
% the maximum capacity
    a.sleaf = a.sub.leaf.max * (c.lleaf.cur/(k.sub.leaf + c.lleaf.cur))^(1)*(1-(c.sleaf.cur/c.leaf.cap))^(1);
```

```
% --------calculatting he cytoplasm and cell wall-bound concentration after local "loss" ----

m.lleaf.cur = c.lleaf.cur/1000 * m.leaf.cur; % [g/g]
m.sleaf.cur = c.sleaf.cur/1000 * m.leaf.cur; % [g/g]
% Express current Cd content in terms of mass; converted from mg/g
% to g/g

aa.diff.leaf1 = a.lleaf*((m.stem.cur + m.stem.new)/2) - a.sleaf*((m.leaf.cur + m.leaf.new)/2);
aa.diff.leaf.rev1 =    a.sleaf*((m.leaf.cur + m.leaf.new)/2) - a.lleaf*((m.stem.cur + m.stem.new)/2);

% local variable that shows the difference between the regional gain and local loss
if (aa.diff.leaf1 > 0)
m.lleaf.postLocal = m.lleaf.cur + (aa.diff.leaf1* dt);
m.sleaf.new = m.sleaf.cur + ((m.leaf.cur + m.leaf.new)/2)*(a.sleaf*dt);
% The new Cd in cytoplasm = current + regional gain - local loss
% The new Cd in cell wall-bound = current + local "loss"
else
m.lleaf.postLocal = m.lleaf.cur;
m.sleaf.new = m.sleaf.cur + (aa.diff.leaf.rev1*dt);
% The new Cd in cytoplasm = current; NO gain because everything is
% gone to the bound form
% The new Cd in cell wall-bound = current + what was available from
% regional gain
end

c.lleaf.postLocal = m.lleaf.postLocal / m.leaf.new * 1000; %[mg/g]
% The new Cd bound is due to local "gain"
c.sleaf.new = m.sleaf.new/m.leaf.new * 1000; % [mg/g]
% Converting the Cd content back in terms of concentration which
% may or may not the dilute the previous concentration depending on
% the growth

%-------------------------------------------------------------------------
c.lleaf.new = c.lleaf.postLocal;

% store concentration information for the time step
c.system(1,tnow) = c.lleaf.cur + c.sleaf.cur; % concentration total for the compartment for current time step
c.lsystem(1,tnow) = c.lleaf.cur; % concentration of each compartment in liquid form for current time step
c.ssystem(1,tnow) = c.sleaf.cur; % concentration of each compartment in solid form for current time step
```

```
m.lsystem(1,tnow) = m.lleaf.cur; % mass of Cd floating in cytoplasm for current time step
m.ssystem(1,tnow) = m.sleaf.cur; % mass of Cd bound to the cell walls/vacuoles for current time step
a.lsystem(1,tnow) = a.lleaf;
a.lsystem(1,tnow) = a.sleaf;
end
end
%-----------------------------------------------------------------------
%------------------------UPDATE MATRIX -------------------------------
%-----------------------------------------------------------------------
%total concentration
c.total= sum(c.system);
%Update time step (i.e. next time step's current = current's new)
c.sol.cur = 5;    %New solution concentration
c.lroot.cur = c.lroot.new; % New root concentration in liquid form
c.lstem.cur = c.lstem.new; % New stem concentration in liquid form
c.lleaf.cur = c.lleaf.new; % New leaf concentration in liquid form
c.sroot.cur = c.sroot.new; % New root concentration in solid form
c.sstem.cur = c.sstem.new; % New stem concentration in solid form
c.sleaf.cur = c.sleaf.new; % New leaf concentration in solid form

m.lroot.cur = m.lroot.new; % New root mass in cytoplasm
m.lstem.cur = m.lstem.new; % New stem mass in cytoplasm
m.lleaf.cur = m.lleaf.new; % New leaf mass in cytoplasm
m.sroot.cur = m.sroot.new; % New root mass bound on cell walls/vacuoles
m.sstem.cur = m.sstem.new; % New stem mass bound on cell walls/vacuoles
m.sleaf.cur = m.sleaf.new; % New leaf mass bound on cell walls/vacuoles

m.root.cur = m.root.new; % New root biomass
m.stem.cur = m.stem.new; % New stem biomass
m.leaf.cur = m.leaf.new; % New leaf biomass
m.total.cur = m.total.new; % New total biomass

 r.stem_root.cur = r.stem_root.new; % New stem to root ratio
 r.leaf_stem.cur = r.leaf_stem.new;% New leaf area to total biomass ratio

% m.stem_root.cur = m.stem_root.new;
%-----------------------------------------------------------------------
%------------------------PLOT-----------------------------------------
%-----------------------------------------------------------------------
```

```
        % Movie
        temp2 = mod(tnow,2);
    if (temp2 == 0)
            v = v + 1; % count the frame
            plot(tstep-tmin, c.lsystem); %Plot
            axis([1 tmax-tmin 0 1.5]);
            Mov (v) = getframe;
    end
end
```