

Hyperaccumulation mechanism in plants and the effects of roots on rhizosphere soil chemistry - A critical review

Kwon-Rae Kim¹, Gary Owens¹, Ravi Naidu^{1,2}, and Kye-Hoon Kim^{3,*}

¹Centre for Environmental Risk Assessment and Remediation, University of South Australia, Mawson Lakes, SA 5095, Australia

²Cooperative Research Centre for Contamination and Remediation of the Environment, University of South Australia, Mawson Lakes, SA 5095, Australia.

³Department of Environmental Horticulture, University of Seoul, Seoul, Korea

Much research has been conducted in the field of phytoremediation since the discovery of the range of plants known as hyperaccumulators. Research has focused simultaneously on elucidating the mechanism of metal(loid) accumulation and development of practical techniques to enhance accumulation efficiency. To date, it is generally understood that there are five specific mechanisms employed by hyperaccumulating plant species that are either not or under utilized by non-hyperaccumulators. These include 1) enhanced metal(loid)s uptake through the root cell, 2) enhanced translocation in plant tissue, 3) detoxification and sequestration, 4) enhanced metal availability in soil:root interface, and 5) active root foraging toward metal(loid) enriched soils. Among these mechanisms, understanding of the plant-root effect on metal(loid) dynamics and subsequent plant uptake is vital to overcome the inherent limitation of phytoremediation caused by low metal(loid) solubility in soils. Plant roots can influence the soil chemistry in the rhizosphere through changes in pH and exudation of organic compounds such as low-molecular-weight organic acids (LMWOAs) which consequently change metal(loid) solubility. The decrease in soil pH by plant release of H⁺ results in increased metal solubility. Elevated levels of organic compounds in response to high metal soil concentrations by plant exudation may also increase metal concentration in soil solution through formation of organometallic complexes.

Key words: Root exudation, Metal(loid)s, Phytoremediation, Plant roots, Metal solubility

Introduction

Contamination of soils by metals and metalloids through anthropogenic and geogenic activities is a widespread and serious problem confronting scientists, regulators and societies worldwide. Unlike organic contaminants, metals and metalloids are non-degradable and tend to accumulate in soils because of their association with organic matter and mineral surfaces (Alloway and Jackson, 1991; Cunningham et al., 1995). The disappearance of metal(loid)s from soils usually indicates they have leached into lower strata and may possibly be released into groundwater or surface/subsurface runoff. For this reason metal(loid)-contaminated sites which have not been remediated or otherwise made safe have the potential to result in surface and/or groundwater contamination. Remediation of metal

contaminated soils is challenging and often extremely expensive. Recently, there has been significant interest in phytoremediation of such soils given its low cost and acceptance by the community as a green technology. However, successful phytoremediation requires a detailed understanding of hyperaccumulation mechanisms. In order to elucidate the physiological and biological mechanisms of phytoremediation, many researchers have conducted studies involving uptake of metal(loid)s through the root, the subsequent transfer of these elements from root to shoot, and the detoxification mechanisms such as complexation with phytochelatins and cellular/subcellular compartmentalization (Lombi et al., 2002; Salt and Kramer, 2000; Whiting et al., 2003; Zhao et al., 2003a).

Despite many recent studies directed towards an understanding of the ability of potential plants for phytoremediation to tolerate and accumulate metals within their tissues (Bert et al., 2003; Boominathan and Doran, 2003; Ebbs et al., 2002; Salt et al., 2000), the

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*Corresponding author: Phone : +82222102605,

E-mail : johnkim@uos.ac.kr

mechanisms involved in metal acquisition and uptake are still poorly characterized. In particular, little is known about the relative contributions of plant synthesized compounds that might affect the efficiency of metal transfer from root to shoot and the processes that occur in the rhizosphere. Moreover the molecular processes controlling the dynamics of contaminants in the rhizosphere is poorly understood and this may be one reason for the current difficulty in enhancing the rate of metal uptake by plants.

Hyperaccumulation mechanisms

A brief survey of the existing literature quickly indicates that in general high uptake mechanisms of metals by hyperaccumulator plants fall into four broad categories: a) enhanced adsorption of metals into the roots (Lasat et al., 1996) followed by b) high translocation rates of metals from roots to the shoots (Chen and Cutright, 2001; Shen et al., 1997) c) detoxification and sequestration of heavy metals in tissues for tolerance (Ebbs et al., 2002; Ryan et al., 1995; Schmoger et al., 2000) d) enhanced availability of metals in the rhizosphere resulting from changes in pH, redox potential, and excretion of organic acids and/or chelates by roots (Hinsinger, 2001) and e) foraging for metals by roots, involving preferential allocation of root biomass into regions of metal enrichment (Schwartz et al., 1999).

Enhanced uptake One of the main mechanisms that enable hyperaccumulators to accumulate such high levels of metal may be simply due to a natural high uptake ability of the plant root. According to Lasat et al. (1996), this ability is associated with the amount of metal transporters in the root. Their study showed that the Zn hyperaccumulator *Thlaspi caerulescens* had a significantly higher uptake rate of Zn when compared to the non-hyperaccumulator *Thlaspi arvense*. The hyperaccumulation was attributed to the plasma membranes of root cells of *Thlaspi caerulescens* having a higher density of Zn transporters. The maximum initial velocity (V_{\max}) for Zn influx in *Thlaspi caerulescens* was 4.5 fold higher than in the nonaccumulator *Thlaspi arvense* (Lasat et al., 1996). A significantly larger V_{\max} suggests that there was a higher density of Zn transporters per unit membrane area in *Thlaspi caerulescens* roots.

Lasat et al. (2000) successfully cloned genes encoding for the high affinity Zn transporter, ZNT1, in *Thlaspi*

caerulescens. They found that ZNT1 was highly expressed in the roots of *Thlaspi caerulescens* regardless of plant Zn status, whereas in non-hyperaccumulator *Thlaspi arvense*, ZNT1 was expressed at far lower levels, and the expression was stimulated by Zn deficiency. Both physiological and molecular studies indicate that *Thlaspi caerulescens* has higher metal adsorption ability than non-hyperaccumulator *Thlaspi arvense*.

Lombi et al. (2001) investigated the difference in adsorption ability between a Cd hyperaccumulating ecotype, *Thlaspi caerulescens*, from Ganges in southern France and a Cd non-hyperaccumulating ecotype, *Thlaspi caerulescens*, from Prayon in Belgium. Their study showed that the V_{\max} for Cd influx was five fold higher in the high Cd ecotype than in the low Cd ecotype. This result also suggests a high-affinity Cd transport system in the Cd hyperaccumulating population of *Thlaspi caerulescens* which enabled it to accumulate a higher level of Cd than the non-hyperaccumulating ecotype. Moreover, Zhao et al. (2002) suggested that the low Cd-accumulating ecotype may take up Cd from soil through Ca channels or transporters for Zn and Mn. However, the high Cd-accumulating ecotype seems to have a highly selective Cd transport system in the root cell membranes.

Even though these studies show the possibility that metal-transporters are in operation in the plant roots, more studies with other hyperaccumulators and metals are necessary for confirmation of metal transporter function.

Translocation The extremely efficient translocation of metals from roots to shoots is one of the key features that distinguish metal hyperaccumulators from non-hyperaccumulators. Hyperaccumulators may simply have a more efficient xylem loading than non-hyperaccumulators. Kramer et al. (1996) found that exposing several Ni hyperaccumulator species of *Alyssum* to Ni resulted in a large and proportional increase in the levels of histidine in the xylem sap, which was shown to be complexed with Ni. Furthermore, it was shown that supplying exogenous histidine to the non-accumulator *A. montanum*, either as a foliar spray or in the root medium, considerably increased the nickel tolerance of this species and greatly increased nickel flux through the xylem.

Salt et al. (1999) studied the existence and influence of metal induced ligands for complexing Zn in root, xylem

sap and leaves of *Thlaspi caerulescens*. In the root, Zn was mainly coordinated with histidine, while in the xylem sap Zn was mainly transported as a free hydrated cation, and in the leaves it was primarily complexed with organic acids. This study indicated that the role of metal-inducing ligands seemed to depend on both metal type and plant species. Therefore, further work is required to ascertain whether metal-induced production of histidine (or perhaps other nitrogen-containing ligands) is found in other groups of nickel hyperaccumulators, or indeed in other hyperaccumulators for other metals.

Detoxification and sequestration in tissue It is well known that metal ions complexed with organic compounds are generally far less toxic than free metal ions. This complexation mechanism typically occurs in plant tissue after plant uptake of metal ions through the root. In non-hyperaccumulators, phytochelatin peptide, synthesised from glutathione, complex with heavy metals such as Cd, Pb and Zn for detoxification (Cobbett, 2000). In hyperaccumulators, phytochelatin peptides are not so important and plant synthesised organic acids seem to be responsible for the detoxification of heavy metals (Cobbett, 2000).

Levels of citric, malic, malonic and oxalic acids have been correlated with elevated concentrations of Ni or Zn in the plant biomass (Lee et al., 1978). In addition, the promotion of metal-organic acid complexes in plant tissue in response to plant exposure to heavy metals has also been indicated using chromatography (Brooks et al., 1981; Kersten et al., 1980; Pelosi et al., 1976; Sagner et al., 1998) and X-ray absorption spectroscopy (Kramer et al., 2000; Salt et al., 1999). The average total concentrations of citric, malic and malonic acids in the roots of hyperaccumulators, *T. caerulescens* and *A. bertolonii*, were 422 ± 53 and $432 \pm 65 \mu\text{mol g}^{-1}$ dry weight, respectively, which is much higher than the normal organic acid concentrations observed in most crop species ($10\text{--}90 \mu\text{mol g}^{-1}$) (Clark, 1969).

In addition to detoxification, binding to the cell wall, localisation in the apoplast, and sequestration in the vacuole may also be involved in mechanisms for metal tolerance and accumulation (Kramer, 1997; Kramer et al., 1996; Kramer et al., 2000).

Enhanced availability of metals in rhizosphere Restricted bioavailability of metals in contaminated soils may be a major limitation of phytoextraction efficiency.

Metals can exist as various different species in the soil including water soluble and exchangeable compounds which can both be easily accessed and utilized by plants, as well as complexed species, which are less well accessed and used by plants because of their low lability. Such non-labile metal ions are not immediately available for uptake by plant roots. However, several studies have indicated that this non-labile metal could be released by the metal mobilizing ability of hyperaccumulator plant roots (root exudation).

Comparison of the changes in the soil solution Zn and Cd concentrations after growth of *Thlaspi caerulescens* in seven contaminated soils from Europe showed that both soluble Zn and Cd decreased considerably at the end of the experiment (Knight et al., 1997). However, decreases in the soil solution pool accounted for only about 1% and 50% of the total Zn and Cd uptake, respectively, by the plants. These results suggested that most of the Zn and approximately 50% of the Cd taken up by *Thlaspi caerulescens* were from a non-labile fraction. This meant that either *Thlaspi caerulescens* was highly efficient at mobilising Zn or Cd which was not initially soluble, or that the soils used had low buffering capacities that were able to replenish the soil solution Zn and Cd within a very short time.

Blaylock and Huang (2000a) also suggest that hyperaccumulators are able to modify the rhizosphere to enhance metal solubility. Their study showed increases in Zn accumulation in the shoot of the non-hyperaccumulator *Thlaspi arvense*, when it was co-cultivated with a Zn hyperaccumulator *Thlaspi caerulescens*.

In contrast studies using isotopic dilution methods to determine the Cd mobilizing ability of the roots of *Thlaspi caerulescens* concluded that *Thlaspi caerulescens* was unable to strongly mobilize Cd from the non-labile pool in the soil (Hutchinson et al., 2000).

Even though several researchers have suggested the possible mobilizing ability of the hyperaccumulator root, a complete understanding of the exudation of phytosiderophores and organic acids, and the resulting changes in pH and redox potential, which are considered key factors in controlling metal availability in the rhizosphere (Lombi et al., 2000), is still poorly understood and further studies will be of great benefit to the field.

Root allocation In order to understand the uptake mechanism of hyperaccumulators, investigations of root morphology of hyperaccumulators in response to

metal(loid) soil concentrations is important. Therefore, investigation of penetration and exploitation of contaminated soils by roots of hyperaccumulators is a prerequisite for understanding and progressing hyperaccumulation technologies.

Studies of the development of roots of the Zn-hyperaccumulator *Thlaspi caerulescens* under various conditions of soil contamination (Schwartz et al., 1999) indicated that the roots of *Thlaspi caerulescens* exhibited two major types of morphology and architecture according to the presence of Zn in the soil a) short roots in clusters when Zn was present at high rates; b) rather finer and longer roots in uncontaminated soil. The quantity of available Zn in the soil was a primary factor controlling the development of roots. The effects of localized metal enrichment on the allocation of the root of Zn hyperaccumulator *Thlaspi caerulescens* have previously been studied using a rhizobox system (Whiting et al., 2000) and showed that plants consistently allocated 70% of their total root biomass and length into the metal-enriched soil. This highlights the unique positive response of the roots of *Thlaspi caerulescens* to a Zn enriched part of soil.

Root allocation of two populations of *Thlaspi caerulescens* differed in their abilities to accumulate Cd in the localized Cd-enriched soil (Whiting et al., 2000). High Cd ecotype from Ganges showed increased root biomass and root length allocation into the Cd-enriched soil. In contrast, low Cd ecotype from Prayon showed no such increase. This result suggests that high metal accumulating ecotypes seem to have a gene responding to the existence of metal to allocate their root for foraging for metal. Despite those studies, there was still lack of information on the other type of metals and plants.

Root influence on the chemistry in the rhizosphere

Characteristics of rhizosphere soils

The concept of a rhizosphere was first proposed by Hiltner in 1904 to describe the volume of soil surrounding a living plant root that is influenced by root activity either directly or indirectly (Hinsinger, 1998; Hinsinger et al., 2005a). Plant root interactions with soil solution chemicals can drive the exudation of synthesized organic compounds and subsequently influence the environment in the vicinity of the roots by stimulation of microorganisms and increased cation solubility (Jones et

al., 2004).

The rhizosphere is a known depletion spot for nutrients (nitrate-N, P and K) occurring as a consequence of the sink-effect of the adsorbing root of higher plants (Hinsinger, 2001; Hinsinger et al., 2005a). The depletion zone for P and K extends from less than 1 mm up to several millimetres while the depletion zone for nitrate-N extends for several centimetres. In addition to this the chemical changes in the rhizosphere such as pH and DOC result in the changes in heavy metal solubility. For instance, Wenzel et al. (2003a) characterized the rhizosphere of the Ni hyperaccumulator, *T. goesingense* under field conditions (Wenzel et al., 2003) and found larger pH, EC, and DOC in the rhizosphere of *T. goesingense* compared to the bulk soil. This change in the rhizosphere chemical nature resulted in enhanced Ni solubility.

Root effect on pH

Acidification mechanism The soil pH changes in the rhizosphere are related to the balance of cation or anion uptake and concomitant release of H^+ / OH^- and organic acids (Sas et al., 2001). Generally, plant roots secrete H^+ when cation uptake exceeds anion uptake and extrude OH^- when anion uptake exceeds cation uptake (Sas et al., 2001). Hence acidification occurs directly by releasing protons from the roots when cations exceed anion uptake (McLay et al., 1997; Van Beusichem, 1981). For example, legumes, reliant on N_2 fixation, generally take up more cations than anions and thus exude proportionally more H^+ than OH^- to balance charge in the rhizosphere (Tang et al., 1997) and consequently lower soil pH.

Another possible biological function of roots affecting the rhizosphere pH is the respiration of both roots and rhizosphere microorganisms. This process contributes a significant build-up of CO_2 in the rhizosphere and ultimately in the bulk soil as the respired CO_2 can diffuse away from the root surface to a significant distance (Hinsinger et al., 2005b). Van Breemen (1991) also reported that soil acidification via biological processes could include the effect of carbon dioxide emitted by micro-organisms and roots and that transformation to H_2CO_3 in soils resulted in pH increases in the range from 5 to 7. Furthermore, several aliphatic and aromatic organic acids formed by micro-organisms during the degradation of lignocellulose and humic substances, or exuded by roots and lysing plant residues may be

dominant acidifying agents (Gramss et al., 2003).

Acidification for hyperaccumulation While it was suggested that hyperaccumulator plants might acidify the rhizosphere soils and subsequently increase the solubility of heavy metals (McGrath et al., 1997; Sas et al., 2001), there was also some evidence that acidification was not involved in the hyperaccumulation mechanism since some researchers had observed that soil pH increased following plant culture (Knight et al., 1997; Luo et al., 2000). In pot experiments using *Brassica Juncea*, soil solution (moisture suction sampler, Rhizon SMS) pH decreased by about 0.5 units within the first 10 days of growth and subsequently, from 10 days to 20 days, the pH decreased more rapidly (Wu et al., 2003). They concluded that rhizosphere acidification could be one of the more important mechanisms by which the *Brassica Juncea* plants mobilized soil nutrients and heavy metals. Furthermore, rhizosphere soils were more acidic than the non-rhizosphere soils, with differences in the range of 0.2-0.4 pH units with the Cd and Zn hyperaccumulator, *T. caerulea* (McGrath et al., 1997). However, in their study, despite the significant higher Zn uptake by *T. caerulea*, the acidification degree was similar to that of the non-hyperaccumulator, *T. ochroleuca*. This indicated that acidification is not a unique mechanism for hyperaccumulators. Bernal et al. (1994) also found no difference between rhizosphere pH of Ni hyperaccumulator *Alyssum murale* and radish (*Raphanus sativus* L.). Even under field conditions, the Ni hyperaccumulator, *T. goesingense* increased soil pH in the rhizosphere compared to bulk soil (Wenzel et al., 2003). These contrasting findings may be due to the different soil and plant types employed in each study and hence further investigation considering the effect of plant and soil type on soil acidification would be of great benefit.

Root exudation

The ability to secrete a vast array of compounds into the rhizosphere is one of the most remarkable metabolic features of plant roots, with nearly 5 to 21% of all photosynthetically fixed carbon being transferred to the rhizosphere through root exudates (Marschner, 1995). Through the exudation of a large range of organic and inorganic substances, plant roots may be governing the environment including regulating the soil microbial community in their immediate vicinity, coping with

herbivores, encouraging beneficial symbioses, changing the chemical and physical properties of the soil, and inhibiting the growth of competing plant species (Nardi et al., 2000).

From early investigations using semi-quantitative techniques, it soon became apparent that the composition of root exudates is highly variable and dependent on plant species, plant age and the physiochemical environment (Curl and Truoglove, 1986). Generally, low molecular weight (LMW) compounds such as amino acids, organic acids, sugars, phenolics, and various other secondary metabolites are believed to comprise the majority of root exudates, whereas high molecular weight exudates primarily include mucilage (high-MW polysaccharides) and proteins (Walker et al., 2003). In addition, it has been predicted that a steep gradient of organic exudates exists in the rhizosphere with an effective sphere of influence in the rhizosphere of between 0.2 and 1.0 mm, depending on soil type, organic acid type and time (Darrah, 1991; Jones and Darrah, 1996).

It has been proposed that hyperaccumulating plant species may enhance metal solubility in the rhizosphere via enhanced root exudation when compared to non-hyperaccumulating plants and subsequently increase metal uptake (Knight et al., 1997). Root exudates could potentially increase metal uptake via indirect effects on microbial activity, rhizosphere physical properties and root growth dynamics and directly through acidification, chelation, precipitation and oxidation-reduction reactions in the rhizosphere (Marschner, 1995; Uren and Reisenauer, 1988). However, exudation rates and the chemical composition of exudates from metal hyperaccumulator species are virtually still unknown and extremely difficult to collect from soil-grown plants (Wenzel et al., 2003).

Dissolved organic carbon (DOC) from roots Plant roots contribute to the DOC concentration in the rhizosphere. For example, in the study by Wenzel et al. (2001) using a slit rhizobox, DOC concentrations increased significantly toward the root plane within a period of 8 days and the accumulation of DOC within 1 mm from the root plane corresponds to about 3.8 mg DOC g⁻¹ root DW in 8 days (Wenzel et al., 2001). DOC in this study comprised other low-molecular-weight organic carbons (LMWOCs), with typical LMWOC compositions of canola root exudates being >50% sugar, 10-40% organic acids, and 10-15% amino acids and amides (Wenzel et al., 2001).

Organic acids (OAs) from plant roots Plants may influence the amounts of LMW carboxylic acids in soils and soil solutions (Cieśliński et al., 1998; Strobel, 2001). For example, while no water extractable organic acids were found in the bulk soil, high concentrations of OAs were detected in the rhizosphere of durum wheat, indicating that the organic acids found in the rhizosphere soil were directly from root exudates and/or microbial metabolites at the soil/root interface (Cieśliński et al., 1998). The most commonly reported organic acids exuded by plants are those that participate in the tri-carboxylic acid cycle including citric, succinic, malic and cis-aconitic acids, together with the aliphatic acids (formic, acetic, propionic, butyric, oxalic, and tartaric acids), aromatic acids (*p*-coumaric, caffeic, ferulic, gallic salicylic) and protocatechuic acids (Jones, 1998; Stevenson, 1967). In particular, acetic, lactic, malic and formic acids are ubiquitous in plant roots and in the rhizosphere soil, ranging from several μM in the plant roots to several hundred μM in the rhizosphere (Han et al., 2005; Li et al., 2003; Strobel, 2001).

Jones suggested that acetic and lactic acids are the highest concentrations of all LMWOAs (Jones, 1998) because these two organic acids are more likely to originate from rhizosphere bacteria and are the most abundant carboxylic acids in soil solution (Li et al., 2003; Strobel, 2001).

However, the abundance of a particular set of OAs are more likely to be dependent on plant and soil type, and the general exudation characteristics of OAs from roots can not always be applicable to all plants and soils. The OAs exuded from durum wheat roots using three soil types found that the total water extractable LMWOAs in the three soils studied ranged from 50.6 to 5520 nmol g^{-1} DW roots (9.4 to 953.6 nmol g^{-1} soil, respectively) and acetic and succinic acids accounted for 93.2%, 88.4%, and 84.2% of the total LMWOAs for the three soils (Cieśliński et al., 1998). High levels of acetic and succinic acids have also been found in root exudates of wheat (Rovira, 1969). This prevalence for acetic and succinic acid exudation is very different from the other studies which show that lactic, malic, and formic acids are the most abundant OAs exuded from roots (Li et al., 2003; Strobel, 2001). While citric and tartaric acids have been reported as the predominant compounds in root exudates of various monocotyledonous species, namely corn and wheat (Elgala and Amberger, 1988; Krafczyk et al., 1984), other studies have reported that both citric

and tartaric acids were either not detected or were present in relatively low concentrations (Cieśliński et al., 1998).

Stimulation for OAs exudation External stimuli may result in enhanced plant exudation. External stimulation or stress of plant roots which promotes OAs exudation can broadly be divided into two categories, nutrient deficiency and metal toxicity. Nutrient deficiency, resulting from P and Fe deficiencies have previously been reported as directly resulting in OAs exudation. In P deficient treatments for lupin, citrate (90%) and malate (10%) were detected as the primary components in root exudates (Sas et al., 2001) while Fe deficiency induced a 5 fold increase in OAs in root tissue and also induced large OAs exudation from the root (Guerinot and Yi, 1994; Landsberg, 1981).

Metal toxicity, resulting from elevated levels of di- and tri-valent metals such as Cu, Pb, and Al induced exudation of OAs from roots of several plant species (Li et al., 2002; Li et al., 2000). Linear increases in citrate release from wheat root with elevated Cu concentration up to 12 μM were found in four crops (rye, triticale, maize and soybean) which secreted both citrate and malate with increasing Cu treatment, suggesting that Cu-induced exudation of organic acids was a common phenomenon in these crops (Nian et al., 2002). Oxalate content was significantly increased in the root or rhizosphere soil of Pb tolerant rice varieties with Pb treatment (Yang et al., 2001) and Al induced efflux of malate was observed in the rhizosphere of wheat roots (Hettiarachchi and Pierzynski, 2004).

Effect of soil type on nature of OAs In general limited research has been specifically conducted to elucidate the role of soil type on metal exudation by plant roots. The effect of soil type on the quantity of organic acids detected in the rhizosphere soil was more pronounced (one to two orders of magnitude difference in total amount of LMWOAs between soils) compared to the LMWOAs production between durum cultivars (Cieśliński et al., 1998). The differences in LMWOAs between soils could be the result of the plants response to varying soil fertility levels possibly affecting root growth and the amount of soil collected from the root surface (Cieśliński et al., 1998). Also, the differences in LMWOAs concentrations in soil could be altered by microbe-root interactions in the rhizosphere soil.

Other compounds from root It is generally acknowledged that an average of about 20% of C assimilated by higher plants via photosynthesis is released by roots as compounds including sugars, polysaccharide mucilage, phytosiderophores, organic and amino acids, peptides and proteins. The percentage of C assimilated can vary from 10% to 50% depending on the plant and soil type (Hinsinger et al., 2005b). Phytosiderophores (amino acids) are generally found in the root exudates of *Graminaceous* plant species and can form much more stable complex with Fe, Zn, and Cu compared to carboxylates (Chaignon et al., 2002; Hinsinger, 1998). In a study with three maize varieties showing differential resistance to Al toxicity, high exudation rates of phenolic catechol, catechin, quercetin and curcumin were observed (Kidd et al., 2001). The amounts released correlated well with the resistance of the varieties to Al (Mariano et al., 2005). In addition, many researchers characterized polysaccharide mucilage from the root exudates of the seedling growing under laboratory conditions (Bengough and McKenzie, 1997; Morel et al., 1986).

Nature and function of root exudates

Root exudates, which consist of a variety of compounds produced by plant roots and by bacterial metabolism, are involved in many physicochemical processes operating in the rhizosphere, including water holding, soil aggregate formation, nutrient acquisition, metal detoxification, and alleviation of anaerobic stress in roots (Jones, 1998; Walker et al., 2003). The following sections summarise the nature and functions of root exudates.

Physical function of root exudates Various functions have been attributed to root exudation. Root exudates could play a major role in the maintenance of root-soil contact, which is especially important to the plant under drought and dry conditions (Walker et al., 2003). Young (1995) found that rhizosphere soil was significantly wetter than bulk soil and suggested that exudates within the rhizosphere increased the water holding capacity of the soil. Root exudates can protect the roots from desiccation, stabilize the soil micro aggregates, and selectively adsorb and store ions (Bengough and McKenzie, 1997; Griffin et al., 1976; Hawes et al., 2000). In particular, alternate wetting and drying of the mucilage secreted from roots plays an important role in the formation of soil aggregation in the rhizosphere due to its

adhesive properties (Morel et al., 1991) as well as smoothing the passage of the root through the soil (McCully, 1999).

Nutrient acquisition Elevated organic exudates from plant roots in the rhizosphere may influence nutrient solubility and uptake indirectly through their effects on microbial activity, rhizosphere physical properties, and root growth patterns, and directly by acidification, chelation, precipitation, and oxidation-reduction reactions (Uren and Reisenauer, 1988).

In particular, LMWOAs improved the weathering and release of cations from soil minerals and rock fragments, and increased the availability of nutrient cations in the rhizosphere of plants and fungi (Hersman et al., 1995; Marschner, 1995). Furthermore, LMW carboxylic acids are known to increase phosphate solubility by dissolving phosphate precipitates and plant available potassium by increased weathering of potassium containing soil minerals (Fox et al., 1990; Hue et al., 1986; Jones, 1998).

Plants also tended to release more organic exudates actively when they were exposed to nutrient deficiencies. This plant reaction is a known mechanism for nutrient acquisition. The high release of phytosiderophores was observed in wheat root under Fe deficiency compared with Fe sufficient plants and subsequently the Fe solubility was increased to the Fe deficient plant resulting in higher uptake (Awad et al., 1994). The increased mobilization of Fe over a distance of up to 4 mm from the central root compartment (rhizobag) demonstrates the high capacity of phytosiderophores for Fe solubilization in the rhizosphere even under non-axenic conditions (Awad et al., 1994). Zinc deficient plants also released more phytosiderophores (18-20 fold more), compared to Zn sufficient plants (Ismail et al., 1996). In addition to phytosiderophores, it was proposed that organic acids from plant roots such as citrate and malate may play an important role in supplying Fe to dicotyledonous plants (Jones et al., 1996). They are potent complexing agents of Fe in soil and induce the dissolution of previously unavailable insoluble ferric oxyhydroxides. Indeed, a 5 fold increased accumulation of organic acids was found in root tissue and this drove the elevated organic acids excretion (Guerinot and Yi, 1994; Landsberg, 1981).

Tolerance and detoxification It is well known that high concentrations of heavy metals are detrimental to plant growth due to their inherent toxicity and adverse effects

on plant physiological mechanisms. However, some plants have specific mechanism to reduce the toxicity of heavy metals through exudation of organic acids. Specific organic acids can sequester heavy metals and protect the roots from toxicity effects (Bert et al., 2003; Ryan et al., 1995; Zhao et al., 2003b).

One well-known mechanism, responsible for Al tolerance in crops, is Al-induced exudation of organic acids. Al-tolerant genotypes or species are able to release more Al specific organic acids than Al-sensitive species, where organic acid release is specifically induced by Al stress (Delhaize et al., 1993; Ryan et al., 1995). Once these organic acids have left the root, they rapidly form Al-organic acid complexes in the apoplast and soil solution, rendering Al non-toxic and making the roots 5-20 times more resistant to Al (Delhaize et al., 1993). In addition, the release of root exudates in response to Al only occurs at the root apex (0-3 mm) which correlates well with this being the primary site of Al toxicity (Kochian, 1995). Malate seems to be the main substance released from roots to reduce the Al toxicity. In the study by Nian et al. (2002), a large amount of malate was released from Al-tolerant wheat, but malate was not secreted from Al-sensitive wheat (Nian et al., 2002). Above Al concentrations of 200 μM , malate release becomes saturated (Ryan et al., 1995). The correlation between relative tolerance of the wheat genotypes to Al and the amount of malate released from their root apices was significant (Ryan et al., 1995) and growth measurements were subsequently conducted to investigate the amelioration of Al toxicity by exogenous malate. In the presence of 3 μM Al alone, relative root growth of an Al-sensitive genotype was reduced to 13% compared to the control. On addition of 10 μM malate to the solution, relative root growth increased to 50%, and 20 μM malate completely alleviated the Al-induced inhibition of root growth (Ryan et al., 1995). The results imply that the Al-stimulated efflux of malate from root apices is involved in a general mechanism for Al tolerance in wheat. It is well established that organic acid-Al complexes are less toxic than free Al to plant roots (Bartlett and Regio, 1972; Hue et al., 1986; Kerven et al., 1991). Exogenous malate treatments also significantly alleviate Cu and La toxicity (Parker and Pedler, 1998).

Pb tolerant rice roots synthesize up-regulated oxalate and secrete this to precipitate Pb thereby reducing Pb root uptake (Yang et al., 2001). Binding of Pb to the carboxyl groups of mucilage uronic acids also restricts uptake of

Pb into the root (Morel et al., 1986). Nigam et al. (2001) suggested a role for organic acids in Cd tolerance in plants with the observation that, in spite of elevated Cd accumulation when treated with organic acids, plants grown in a Cd contaminated soil exhibited non-toxic behaviour.

Availability of metal and its uptake by plant Various LMWOAs were able to influence the solubility of heavy metals through the formation of metal-organic complexes (Qin et al., 2004). Feng et al. (2005) suggested that due to the effect of LMWOAs in the rhizosphere, the bioavailability of heavy metals in the rhizosphere is different from that in bulk soil (Feng et al., 2005) and this mechanism might be associated with the hyperaccumulation mechanism (Knight et al., 1997). In addition, there is increasing evidence that soluble root exudates increase the solubility of metals in the rhizosphere depending on plant species and cultivar. For example, the solubility of Cd, Cu, and Mn were markedly influenced by soluble root exudates from *Z. mays* L. and *Nicotiana* spp (Mench and Martin, 1991). Organic acids were also very effective in continuously releasing Cd from the natural soils when renewed every 2 h, indicating the importance of the dynamics of LMWOAs in the mobility of Cd at the root:soil interface through the formation of soluble Cd-LMWOA complexes (Onyatta and Huang, 2003).

Increased metal solubility subsequently influences plant metal uptake. Cieřliński et al. (1998) found that higher total amounts of LMWOAs in the rhizosphere soil of the Kyle cultivar (Cd accumulating cultivar) correlated better with higher Cd concentrations in the plant tissue compared to Arcola (Cd non-accumulating cultivar). This suggested that these higher levels of LMWOAs were responsible for greater Cd availability in the rhizosphere and subsequent accumulation of Cd in the Kyle cultivar (Cieřliński et al., 1998).

Another possible role of organic acids is to increase the metal accumulation in plant tissue through enhancing metal translocation. The limited long-distance translocation of a metal ion is one of the main reasons for unsuccessful hyperaccumulation, probably due to the binding of cations to exchange sites located in the xylem cell walls (White et al., 1981). The possible formation of metal chelates or complexes with organic acids from roots in soils, however, may result in easy availability of soil Cd and effective transport of Cd-organic complexes in

plants (Seden and Wolterbeek, 1990). Indeed, Cd added with organic acids resulted in statistically significant increases in Cd accumulation in root and aerial parts of the plant in both sand and soil cultures (Nigam et al., 2001).

Conclusion

Generally, hyperaccumulator plants show dramatically higher metal accumulation than non-hyperaccumulation plants, implying that there would be specific mechanisms in hyperaccumulators responsible for elevated metal uptake. These potential mechanisms include enhanced metal uptake and translocation, detoxification and sequestration in plant tissue, enhanced metal availability in the soil:root interface, and root foraging toward metal enriched soils. Among those mechanisms some researchers emphasised the root reactions on heavy metals in the rhizosphere at the soil:root interface. In particular changes in soil chemical properties, such as pH, OAs, and DOC concentration, induced by plant roots could each significantly influence metal dynamics in the rhizosphere and subsequent metal uptake. However, limited studies have been conducted to investigate the underlying basis for changes in rhizosphere chemistry at a molecular level and the subsequent metal uptake. For these reasons further studies aimed at investigating the underlying basis for physicochemical changes in the rhizosphere are recommended to elucidate the role of these in plant uptake.

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고축적식물의 중금속 흡수기작과 뿌리에 의한 근권 토양의 화학변화 - 총설

김권래¹ · Gary Owens¹ · Ravi Naidu^{1,2} · 김계훈^{3*}

¹CERAR, University of South Australia, Australia, ²CRC CARE, University of South Australia, Australia, ³서울시립대학교 환경원예학과

토양중 중금속을 흡수해서 체내에 고농도로 축적할 수 있는 식물, 이른바 고축적식물(hyperaccumulator)의 발견으로 오염토양에 대한 식물복원(phytoremediation) 기술에 대한 많은 연구들이 수행되고 있다. 이들 연구의 방향은 크게 고축적식물의 중금속 축적 기작을 밝히기 위한 것과 축적효율을 높임으로써 복원 효율을 향상시키는 실용적인 기술개발로 나누어진다. 지금까지 고축적식물에 의한 중금속 축적 기작은 다섯 가지의 특이 기작으로 알려져 있는데, 1) 뿌리세포의 중금속 흡수 증진, 2) 식물체 조직내의 중금속 이동성 향상, 3) 중금속의 무독화(detoxification) 및 격리(sequestration), 4) 토양-뿌리 경계면에서의 중금속 유효도 증진, 그리고 5) 중금속 오염토양으로의 능동적인 뿌리의 성장 등이 이에 속한다. 일반적으로 토양 중 낮은 중금속 유효도는 식물복원 기술의 현장 적용에 있어 제한요소로 간주된다. 이를 극복하기 위해서는 위에 기술된 다섯 가지 기작 중 고축적식물의 뿌리가 근권 토양중 중금속의 화학변화에 미치는 영향을 이해하는 것이 매우 중요하다. 식물 뿌리에 의한 근권 토양의 pH 변화와 뿌리에서 나오는 분자량이 적은 유기산(low-molecular-weight organic acids, LMWOAs)과 같은 유기성 분비물은 근권부 토양의 화학적 특성을 변화시키고 결과적으로 중금속의 유효도를 변화시킨다. 예를 들어 뿌리에서 나오는 H⁺ 이온은 토양 pH를 감소시키고 이에 따라 중금속의 유효도는 증가한다. 또한 고농도의 중금속에 노출된 뿌리는 많은 양의 유기물질을 분비하게 되고 근권 토양에 축적되는 이 유기물질은 토양중 중금속과 결합하여 유기복합물질(organo-metallic complexes)을 형성하면서 유효도를 증가시킨다.