

Root excretion and plant tolerance to cadmium toxicity – a review

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ABSTRACT

Significant quantities of Cd have been added to soils globally due to various anthropogenic activities, posing a serious threat to safe food production and human health. Rhizosphere, as an important interface of soil and plant, plays a significant role in the agro-environmental system. This article presents a review of relationship between root excretion and microorganisms and plant resistance to Cd toxicity and possible mechanisms. Root exudates markedly altered in species and quantity under Cd stress. Root exudates can affect Cd absorption by plants through changing the physical and chemical characteristics of rhizospheres. The influence of root exudates on Cd bioavailability and toxicity may include modifying the rhizosphere pH and Eh, chelating/complexing and depositing with Cd ions, and altering the community construction, the numbers and activities of rhizospheric microbes. In this paper, the methods to reduce the transfer of Cd in soil-plant system by adjusting rhizosphere environment are discussed, and some aspects are also proposed that should be emphasized in the future research work.

Keywords: bioavailability; cadmium; microorganisms; rhizosphere; root excretion

Cadmium (Cd) is one of the most deleterious trace heavy metals both to plants and animals. With the development of modern industry and agriculture, Cd has become one of the most harmful and widespread pollutants in agricultural soils, and soil-plant-environment system mainly due to industrial emission, the application of Cd-containing sewage sludge and phosphate fertilizers and municipal waste disposal (Davis 1984, Gupta and Gupta 1998, Wu et al. 2003, 2004, 2005b, Lima et al. 2006). In humans, Cd accumulates mainly in the kidney with a biological half-life about 20 years, and leads to pulmonary emphysema and renal tubular damage (Ryan 1982). Extreme cases of chronic Cd toxicity can result in osteomalacia and bone fractures, as characterized by the disease called Itai-Itai in Japan in the 1950s and 1960s, where local populations were exposed to Cd-contaminated

food crops, principally rice. According to recent soil survey done in China, at least 13 330 ha of farmland in 11 provinces were contaminated by varying degrees of Cd (Zhang and Huang 2000); for instance, soils in Zhangtu of Sheyang provinces were highly contaminated with metals, and Cd concentrations in soils and in rice grains were as high as 5–7 mg/kg and 1–2 mg/kg, respectively, both being well above the maximum allowable limits set for soil (Kabata Pendias and Pendias 1992) and cereal food (WHO 1972). Furthermore, Cd contamination is a non-reversible accumulation process, with the estimated half-life in soil varying between 15 and 1100 years (Kabata Pendias and Pendias 1992), and high plant-soil mobility to be easily accumulated in plant tissues, while high accumulation of Cd in plants not only badly affects crop yield and quality, but also gives rise to

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a threat on human health via food chain. In brief, Cd contamination in soil has posed a serious issue to sustainable agriculture and human health worldwide (Davis 1984, Obata and Umebayashi 1997, Athur et al. 2000, Wu et al. 2005a).

Under Cd stress, tolerant species and genotypes in plant kingdom could reduce Cd activity to alleviate or eliminate its toxicity through regulating the physiological and biochemical metabolism. In order to survive, plants have to develop efficient and specific heavy metal detoxification mechanism in different plant species (Punz and Sieghardt 1993). One of the main Cd tolerance mechanisms is involved in depressing Cd bioavailability in soils, thus reducing the amount of Cd uptake. Rhizosphere is an important environmental interface connecting plant roots and soil. Roots excrete some organic substances to rhizosphere during the growth, and rhizosphere controls the entrance of nutrients, water and other chemicals, beneficial or harmful, to plants. Moreover, root rhizosphere, especially under heavy metal stress, is widely different from its original bulk soils. Some motile physiological changes would take place, when plants are grown under heavy metal conditions, and then make a series of physical and chemical reactions of heavy metals in rhizosphere environment to affect their transfer in soil-plant system, which may be beneficial to decrease the metal availability and its absorption by plants. Therefore, it is understandable that the study of root rhizosphere has been one of the most important issues in pollution ecology. This paper reviews the progress of researches on the relationship between root excretion and plant resistance to Cd toxicity. Meanwhile, some aspects such as methods to reduce the transfer of Cd in soil-plant system are also discussed and should be emphasized in the future research work.

Rhizosphere pH and Cd bioavailability and toxicity

A major factor governing the toxicity of a metal in soil is its bioavailability. Bioavailability is considered as the fraction of the total contaminant in the interstitial water and soil particles that is available to the receptor organism. Fischerová et al. (2006) and Tlustoš et al. (2006) investigated As, Cd, Pb, and Zn remediation possibilities on medium contaminated soil and observed that the elements found in plant biomass depend substantially on the availability of these elements in the

soil. Vysloužilová et al. (2003) reported that seven willow clones showed different abilities to remove Cd and Zn, which was dependent on soil type and contamination level, and clones planted in moderately contaminated soil achieved the highest Cd removal, while remediation factors were determined less than 1% for Zn in the heavily polluted soil and also unsatisfactory for Cd. Bioavailability of Cd is largely governed by (i) soil type and its physical and chemical characteristics such as soil pH and redox potential (Eh), (ii) Cd speciation or the nature of Cd applied, and (iii) nature of microorganisms. Furthermore, rainfall, evaporation, and plant transpiration can change trace element concentrations in soil solution (Fischerová et al. 2006). It has been demonstrated that some plants can actively or passively change H^+ excretion under heavy metal stress. Such root-induced changes of rhizosphere pH play a major role in the bioavailability of many pH dependent nutrients, but also potentially toxic metals and a range of trace metals (Hinsinger et al. 2006). Tu et al. (1989) observed that Cd has an inhibitory action on H^+ excretion, and proton pump activity decreased by 60% when plants grew in the solution with 50 μM Cd. Proton pump is important for providing energy in the uptake and movement of ions through plasma membrane. Solubility of most heavy metals is influenced by soil pH. In general, with the decrease in pH, solubility and activity of heavy metals increase. Xian (1989) observed a marked increase in exchangeable Cd (free ion Cd^{2+}) in soil, when soil pH decreased from 7.0 to 4.55, and a decrease in Cd complex with carbonate, and with no changes in organic Cd form. Meanwhile, it is well proved that Cd uptake and accumulation are closely related to the amount of exchangeable Cd, rather than the total Cd (Xian 1989, Wu and Zhang 2002, Cheng et al. 2004). Soil pH significantly affected the accumulation of Cd in strawberry (Szteke and Jedrzejczak 1989) and barley (Wu and Zhang 2002), and there was a significantly negative correlation between fruit Cd content and soil pH under lower Cd levels. Meanwhile, uncontaminated low fruit Cd content was obtained even if grown in Cd polluted soil, but with high pH. Yang and Yang (1996) also found a significantly negative correlation between soil pH and plant Cd content. Soil pH affected plant Cd absorption capacity so as to depress the transfer ability of Cd to plants. Cadmium absorption capacity of plants increased three fold along with per pH unit increase in the value of pH 4.0–7.7 (Yang and Yang 1996). Thus, maintaining relatively midpoint rhizosphere pH

of pH 7 may effectively lessen the rapidly available Cd concentration and decrease its deleterious effects. Helmisaari et al. (1999) reported that the addition of lime into pinaster rhizosphere could significantly reduce the solubility of Cu, Ni, Fe, Mn, Pb, and Cd ions in soils. Lime application in acid soil may reduce grain Cd content of wheat by 50% (Lu et al. 1992), and decrease the absorption of Cd in cabbage by 43% (Yang and Yang 1996). Hence, it may be feasible to alleviate Cd toxicity in Cd-contaminated acid soil to elevate pH by lime or lime substances application. Nevertheless, the alteration of pH does not affect plant Cd absorption when in hydroponic experiments; moreover, some studies show that in hydroponic experiment decreasing pH value may result in competition between H^+ and Cd^{2+} , and thereby reducing Cd absorption (Lu et al. 1992).

Rhizosphere oxidation/reduction conditions and Cd bioavailability and toxicity

Plant root rhizosphere redox potential (Eh) differs from that of bulk soil. For example, rice rhizosphere Eh is constantly higher than non-rhizosphere Eh because of the special O_2 secretion of rice roots, which made the most of Fe^{3+} and Mn^{4+} adsorbed to root surface so as to prevent excess absorption by plants. Contrarily, xerophil rhizosphere Eh is usually lower than that of bulk soil, mainly for the following reasons: (i) roots consume oxygen, (ii) roots excrete reducing substances (e.g. phenolics) which can react with Fe^{3+} or Mn^{4+} , (iii) rhizosphere microorganisms must consume oxygen when they make use of root secretion. Meanwhile, the changes of rhizosphere pH may affect the value of Eh, and also the soil nutrient status and heavy metal stress significantly influence rhizosphere Eh. Moreover, chemical reactions of heavy metals, especially redox reactions, may be strongly affected by rhizosphere Eh. Eh affects electric charge of certain expandable minerals that may alter their cation exchange capacity (CEC). Under low Eh in soil, H_2S is produced and then Cd reacts with S^{2-} forming insoluble CdS; thus Cd is not easily absorbed by crops. Meanwhile, the presence of plentiful Fe^{3+} and Mn^{4+} is competitive with Cd^{2+} , thereby reducing plant absorption. For instance, Cd absorption and accumulation was significantly reduced with diminishing Eh in reductive conditions formed by flooding rice fields (Lu et al. 1992). Application of $(NH_4)_2SO_4$ and sulphate fertilizers would induce vulcanization in

soil to produce much H_2S , which contributes to formation of CdS. Hassan et al. (2005) also found that the toxic effect of Cd on rice varied with the form of nitrogen fertilizer, and application of $(NH_4)_2SO_4$ to Cd stressed rice plants, compared to NH_4NO_3 or $Ca(NO_3)_2$, would be beneficial to mitigate detrimental effect of Cd and to reduce Cd accumulation in plants.

Deposition/chelation and Cd bioavailability and toxicity

There are some links of ligands, capable of complexation or chelation with heavy metal ions, in natural environment of soils and water. Generally, about half of photosynthates in plants is retransported to roots and some of them (about 12–40%) would be released to rhizosphere during plant development as exudates: sugars and polysaccharides, organic and amino acids, peptides and proteins (Lin et al. 2003, Hinsinger et al. 2006). Root secretion includes organic ligands (e.g. carbohydrates, organic acids, humic acids, polypeptides, proteins, amino acids, nucleic acids, etc.) and inorganic ligands (e.g. Cl^- , SO_4^{2-} , NH_4^+ , CO_3^{2-} , PO_4^{3-} , etc.). These substances functions not only as the energy source of microorganisms, but also as ligands to be chelated with heavy metal ions and then influence pH and Eh conditions as well as chemical characteristics in the rhizosphere. One of the mechanisms by which plants are able to reduce toxicity from heavy metals in soils is the exudation of organic acids. From the range of carboxylates exuded in the rhizosphere, malate, citrate and oxalate are expected to have the most dramatic effect due to their implication in the complexation of metals (Hinsinger 2001). Specific organic acids can sequester heavy metals and protect the roots from toxicity effects (Jones et al. 2003, Jung et al. 2003, Liao and Xie 2004, Schwab et al. 2005). Accordingly, the composition and quantity of root secretion may affect the present form of heavy metals. Graminaceous plant species (e.g. paddy rice) secrete phytosiderophores (amino acids) that can form much more stable complexes than carboxylates with Fe, Cd, Zn and Cu (Römheld 1991, Hinsinger 1998, Chaignon et al. 2002, Xu et al. 2005). Meach and Martin (1991) reported that low molecular weight of organic acids secreted by roots played an important role in solubility and availability of heavy metals, and Cd^{2+} availability would be reduced if Cd^{2+} bound into Cd chelate complex with root secretion. The

roots of some plants, such as wheat and buckwheat, excrete such organic acid (e.g. oxalic acid, malic acid and citric acid) that can chelate with Cd^{2+} to prevent its entrance into roots. Tang (1998) observed that amino acid could also reduce the toxicity of metal ions. In addition, the combination of organic phosphate acids and Cd ions would produce complexes unavailable to plants. Furthermore, root secretion may precipitate pollutants outside the roots by absorbing and embedding them. Lin et al. (1998) found that Cd combined as complex with oxides of Fe and Mn, and with some organic acids was accumulated in rice rhizosphere much more than that in non-rhizosphere soil. Lin et al. (2003) also studied the interaction between root exudates and heavy metals (Pb and Cd) using wheat and rice exudates produced from sterile and non-sterile hydroponic conditions, and the capillary electrophoretic analysis showed considerable differences in root exudates from wheat and rice plants stressed with Pb and Cd, when compared to those treated with no Cd and/or Pb. Equilibrium dialysis demonstrated that root exudates bound metals to an extent that depended on the metal involved; as for wheat exudates, the importance of the binding followed the order $\text{Pb} > \text{Cd}$. Hence, root exudates would influence Cd and Pb absorption and distribution in plants.

Microorganisms induced by root secretion and Cd bioavailability and toxicity

Microorganisms play an important role in the environmental fate of toxic metals including Cd with physicochemical mechanisms affecting transformations between soluble and insoluble phases (Vivas et al. 2006), although they cannot degrade Cd in soil. Such mechanisms are important components of natural biogeochemical cycles for metals. Variations in the chemical behavior of metal species, as well as the composition of microbial cell walls and extracellular materials, can result in wide differences in bioabsorptive capacities (Gadd 2000). Microorganisms participate in various chemical reactions of specific heavy metals by (i) decreasing Cd solubility in the soil via changing rhizosphere pH, metal valence; (ii) formation of insoluble metal sulfides by releasing H_2S ; (iii) producing numerous organic substances; (iv) binding/sequestration of the toxic metal via the cell walls and mucous layer of cell surface of the microorganisms, or by proteins and extracellular polymers etc., and other mechanisms

(Francis 1990). Therefore, microorganisms mainly transform the present states/forms of heavy metal ions to interfere their availability. Chanmugathas and Bollag (1987) systematically investigated sequestration and activation effects of microorganism on the availability of soil Cd, and found that there was a process of sequestration, followed by activation to influence Cd bioavailability. Rayner and Sadler (1989) demonstrated complexation of Cd to polyphosphate granules in the cell membrane and intracellular Cd binding proteins in a tolerant strain of *Pseudomonas putida*, grown in a defined medium containing 3mM Cd. Zhang and Huang (2000) reported that some microorganisms could excrete organic substances capable of chelating and decomposing pollutants under the stress of environmental contamination, and these organic substances assisting with mucus (plant root excretion, e.g. amylose) can form pectin layer covering over root surface, and the expansion of the layer would sequester metal ions outside the roots. Mucus excreted by epiphyte cells and polyphosphate and organic acid in epiphyte tissues can chelate heavy metal ions including Cd, and thus reduce the uptake and transportation of heavy metal ions to shoots. Davies et al. (2001) tested the ability of the arbuscular mycorrhizal fungus, *Glomus intraradices*, to enhance Cr uptake and plant tolerance on the growth and gas exchange of sunflower, and found that mycorrhizal colonization enhanced the ability of sunflower plants to tolerate and hyperaccumulate Cr.

The presence of heavy metals can significantly affect the microbial activities in soil, thus potentially altering the ecology in soil (Suhadolc 2004). Evidence suggests a change in the genetic structure of the soil microbial community under long-term metal stress (Giller et al. 1998). Such adverse effects impacted by Cd can lead to a reduction in biodiversity and resultant functions in the soil. A gradual change in the microbial community structure was noticed in laboratory-incubated soils amended with Cd (Frostegard et al. 1993, Griffiths et al. 1997). Microorganisms within species of the same genus or within strains of the same species can differ in their sensitivity to metals. Giller et al. (1993) demonstrated that *Rhizobium meliloti* was less sensitive, in terms of growth, to Cd than *R. leguminosarum* and *R. loti*. The symbiosis of mycorrhizal fungus and plant roots produce mycorrhiza, which can effectively decrease metal toxicity to plants. Meanwhile, the microbial strains isolated from Cd-contaminated soils are more tolerant to Cd and have developed resistance (Vivas et al.

2003). On the other hand, soil disestablished due to the presence of high amounts of metals usually produce changes in the diversity and abundance of the population of mycorrhizal fungi (del Val et al. 1999), which may expect to interfere with the possible beneficial effects of this symbiotic association. Moreover, some cell walls in microorganism have the capability of binding pollutants. The capability may be attributed to chemical components and structure of its cell walls. In this respect, an important mechanism is the synthesis of extracellular polymeric substances, a mixture of polysaccharides, mucopolysaccharides and proteins that can bind significant amounts of potentially toxic metals and entrap precipitated metal sulfides and oxides. In bacteria, peptidoglycan carboxyl groups are main cationic binding sites in Gram-positive species. Chitin, phenolic polymers, and melanins are important structural components of fungal walls and are also effective biosorbents for metals and radionuclides. For example, *bacillus* can sequester metal ions because of a thick layer of netty peptidoglycan structure in cell walls. In addition, on the surface there are teichoic and teichuronic acids linked to netty peptidoglycan, and due to carboxyl group of teichoic acid, the cell walls carry negative charges and have the function of ion sequestration (Huang 1992). The application of this microbe may be considered as a biotechnological tool to reduce Cd uptake and accumulation in plants grown in moderately polluted soil; it is of a great economical and ecological relevance. Li et al. (1998) also demonstrated various capacities of yeasts in absorbing Cu^{2+} , Cd^{2+} and Ni^{2+} . Yang et al. (2005) reported that the addition of 2 or 5 mg Cd/kg soil could stimulate the microbial activity in soil, while inhibitory influences induced at high Cd level of 10 or 20 mg/kg soil. The addition of low Cd pollutant increased microbial activity in rhizosphere due to the increased secretion of carbohydrate and organic acids in root exudation, which increase the carbon allocation in rhizosphere soil. Whereas a decline of the enhancement in rhizosphere was observed in high Cd treatments owing to critical inhibition on root growth and metal activation in rhizosphere, which strengthen the toxic impact of metal pollutants on soil microbes. There is a need for more researches on changes in species diversity and microbial adaptations in relation to Cd pollution.

Microorganisms do not affect precipitation, however, vulcanization bacteria can produce H_2S , which may react with Cd to form CdS , resulting in a reduced availability of Cd in soil.

The regulation of Cd bioavailability and transfer in soil-plant system

Rhizosphere, as an important interface of soil and plant, plays a significant role in the agro-environmental system (Wang et al. 2002), in which physical and chemical characteristics of soil and biomass activity and community structure of microorganisms are highly influenced by specific physiochemical and biological characteristics prevailing in this habitat (Sørense 1997). Biochemical process in soil could be significantly affected by environmental pollutants including Cd, and consequently lead to a further influence on soil nutrient status and rhizosphere chemistry of Cd and its transferring efficiency in food chain. Controlling or at least predicting the biological and physiochemical conditions will encourage the development of stable rhizosphere conditions with an enhanced capacity to relieve heavy metal contamination. Rhizosphere environment may be adjusted artificially by (i) applying different fertilizers so as to change rhizosphere pH, (ii) water management to change Eh status in soil, (iii) applying organic fertilizer and (iv) using fungicides or inoculating special microbes to regulate and control microbial community in rhizosphere (Davies et al. 2001). However, the manipulation of beneficial combinations of microorganisms depends on a proper understanding of the ecosystem in order to apply a suitable selection of microbes (Díaz et al. 1996). Increasing soil pH through applying alkaline fertilizers can reduce Cd bioavailability, thereby decreasing Cd absorption by plants. Kuo et al. (1984) reported that liming had a remarkable effect in reducing Cd uptake in the Cd-contaminated soil. Cd content in grains of rice, wheat and maize reduced by 55.9%, 34.6% and 21.0%, respectively, when Cd-contaminated soils were amended by liming together with the application of Ca, Mg and P fertilizers (Wang and Wu 1995). The change of cropping system from upland crops to paddy fields will reduce soil Eh and Cd availability. Organic fertilizers not only improve soil fertility, but also provide bioactive substance and energy to soil microorganisms; accordingly they infect soil Cd bioavailability indirectly to decrease Cd biotoxicity. However, some organic fertilizers such as composts made of waste products, containing a certain amount of heavy metals, may result in soil contamination. In addition, heavy metal toxicity may be alleviated by forming insoluble compounds. For example, the application of phosphate can increase soil P content and precipitate

Cd as $\text{Cd}_3(\text{PO}_4)_2$. It thus offers certain realistic significance on safety crop production by artificially regulating and controlling rhizosphere environment to depress Cd absorption and accumulation in plants. Furthermore plants, and especially the tolerant genotypes, would alter excretion characteristics to adjust themselves to heavy metal stress so as to allow safe uptake of metal ions needed in their cytosol and organelles and simultaneously protect themselves against metal poisoning; it is so called biofeedback regulation capacity or 'adaptive stress response', as reported by Zenk (1996). Some specifically tolerant plants, on the other hand, may be potentially used for phytoimmobilisation purposes to decrease the mobility and bioavailability of pollutants via altering soil factors that lower pollutant mobility by formation of precipitates and insoluble compounds and by sorption on roots. Hence, researches on the mechanism of biofeedback regulation would contribute to resistant cultivar selection and breeding.

In summary, researches on plant root secretion and plant resistance to Cd toxicity are of theoretical as well as applied significance. Root exudates can affect metal absorption by plants through changing the physical and chemical characters of rhizospheres. The modes of root exudates in removing heavy metals or reducing accumulation in plants broadly vary, for example by modifying the pH of the rhizosphere, chelating, complexing and depositing with heavy metals, or altering the numbers and activities of the community of rhizospheric microbes. The understanding of Cd-related molecular events is therefore crucial for elaborating the efficient and targeted protection against metal toxicity for all living organisms. Emphasis should be laid on process-oriented correlations between contaminants and root exudates of crops and other plants of Cd resistance and low Cd accumulation with high yield and good quality suitable to be planted in moderately contaminated soils; reversely, specific plants used as Cd-hyperaccumulators should be tested for their efficiency in removing heavy metals in lab and/or field trials. If so, more effective approaches to ecological restoration of polluted soils can be developed and put into practice. For example, we can attempt to plant hyperaccumulators on a large scale in badly contaminated sites, and supplement these sites with additional specific microbes or chelators to enhance the efficiency of phytoremediation. The important ecological contribution and wide perspectives in field application of root exudates provides entirely new research area. Combined and integrated studies of pollution

and chemical ecology, bringing new findings and breakthroughs, are to be expected.

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