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#### Article abstract

Estimating maximum metal concentrations for soils that protect both plant and human health is difficult, because plants can influence the amount of metal that is absorbed from soils or foods. Plant root accumulation of Cd, as well as translocation to shoots, is determined by internal physiological processes, and is not dependent on the free ion concentration of Cd in the growing medium. Absorption of Cd from edible plants by the kidney orliver is proportional to the dose, and is only slightly dependent on the form of Cd in the diet. Plants dominate the biomass (and thus the diets) in many ecosystems; their physiological processes must be considered in efforts to understand the transfer of metals among compartments of the environment.

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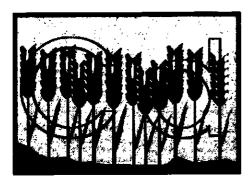
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# **Plants as Modifiers** of Cadmium **Bioavailability**

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#### SUMMARY

Estimating maximum metal concentrations for soils that protect both plant and human health is difficult, because plants can influence the amount of metal that is absorbed from soils or foods. Plant root accumulation of Cd, as well as translocation to shoots, is determined by internal physiological processes, and is not dependent on the free ion concentration of Cd in the growing medium. Absorption of Cd from edible plants by the kidney or liver is proportional to the dose, and is only slightly dependent on the form of Cd in the diet. Plants dominate the biomass (and thus the diets) in many ecosystems; their physiological processes must be considered in efforts to understand the transfer of metals among compartments of the environment.

### RÉSUMÉ

L'estimation des concentrations maximums des métaux dans les sols qui assurent et la santé des plantes et celle des humains est une entreprise difficile, parce que les plantes peuvent influer sur la concentration des métaux absorbés à partir des sols ou des aliments. La concentration

de Cd à partir des racines des plantes, de même que leur translocation dans les pousses est fonction de mécanismes physiologiques internes, mécanismes qui ne dépendent pas de la concentration en ions libres dans le médium de culture. L'absorption du Cd par les reins ou le foie est proportionnel à la dose, et ne dépend que légèrement de la forme du Cd présent dans la diète. Dans beaucoup d'écosystèmes, les plantes constituent la portion principale de la biomasse (et donc de la dière); leurs processus physiologiques doivent être pris en compte dans tout projet visant à comprendre les transferts de métaux d'une portion à l'autre de l'environnement.

## INTRODUCTION

Metals are ubiquitous in our environment. They are in the air, the soil, the water, and therefore they are in biota too; many metals are physiologically essential elements. Plants are the dominant organisms in many terrestrial ecosystems, and they are the critical link between metals in soils and metal exposure of fauna; this is true for both essential and non-essential metals. Yet, the effect of plant processes on the form and fate of the metals is often not taken into account in efforts to predict the movement of metals from soil into biota. Internal and external plant processes can modify the amount of metal that is apparently "bioavailable" in soil. This plant-modification of "bioavailable metal" is relevant to both the amount of metal that moves from soil to plants, and the absorption of metals from edible plants during digestion.

These effects on form and fate mean that the apparent dose, which is measured as total metal in soils or food, is unlikely to be a good estimate of the dose that is finally delivered to the target plant cells or consumers of plants. Since the biological response will be related to the dose at the target site, ecological risk assessments for metals in soils or foods will be more accurate (thus more useful) if metal bioavailability is taken into account when calculating exposure. Estimating the bioavailable fraction of metals in an exposure source is not (yet) a simple matter of applying a predictive algorithm. Ideally, for application to ecological risk assessment, such predictions would be relatively independent of biological

activity. However, the role of plants in moving and modifying metals relative to soil-plant and plant-consumer relationships may be quite significant. This paper describes highlights of various experiments that are investigating the role of plants in metal bioavailability and cycling. The goal of the research program is to gain insight into the mechanisms by which plants play a role in the transfer of cationic metals from soils to important ecological targets.

June 2001

# **UPTAKE AND ACCUMULATION** OF METALS BY PLANTS Suitability of the Free Ion Activity Model for **Predicting Metal Bioavailability**

The bioavailability of metals in soils or soil water has been the subject of intense scrutiny, and many single-step and sequential extraction schemes have been tried and tested, each of which divides the total metal in a soil into categories ranging from highly labile to virtually insoluble. The Free Ion Activity Model (FIAM) of metal bioavailability to organisms is based on the assumption that only the free ions are taken up by organisms, due to the binding specificity of most membrane transporters (Morel and Hering, 1993). While the measurement of the concentration of metal free ion in soil solution (at low environmental concentrations) presents a number of challenges in itself, biotic processes can affect the free ion concentration, particularly in the microenvironment around membranes. As part of normal physiology, plants exude macromolecules (low molecular weight organic acids, peptides, siderophores) and release protons which assist in the acquisition of essential nutrients. The rhizosphere itself is not large, but it does have significant potential for influencing metal free- ion concentration independently of the concentration in soil solution. Exceptions to FIAM have been noted in some biologic applications; the reasons for these exceptions need to be explored before FIAM is accepted or rejected for regulatory use.

The effect of a known root exudate, citrate, on Cd bioavailability to plant roots was examined using durum wheat cultivars (Berkelaar, 2000). Seedlings were placed into solutions containing carefully characterized and controlled concentrations of citrate, Cd(NO<sub>3</sub>)<sub>2</sub>, and essential macronutrients required by

plants. The free ion concentration of Cd was predicted (using MINEQL+) and measured (using ion exchange resin), and Cd accumulation in root tissues, as a function of exposure duration and Cd2+ concentration, was determined (Fig. 1). Citrate enhanced the accumulation of Cd beyond that which would have been predicted from the free ion concentration alone (Fig. 1B), suggesting that either Cd-citrate was being taken up by the roots, or that citrate had an influence on the diffusion of Cd from the bulk solution to the root surface. Calculation of the diffusion rate through the boundary layer from physical parameters suggested that it was smaller or similar in magnitude than the rate of flux into the roots. These calculations required a number of assumptions, so we have concluded (with some caution) that the rate of diffusion of Cd2+ through the solution could indeed have limited uptake and accumulation of Cd by the roots (violating one of the assumptions of the FIAM), and that under this condition, the concentration of Cd2+ in the bulk solution is not a good predictor of bioavailable Cd. This conclusion was arrived at using low concentrations of Cd2+ in the bulk solution, and rapidly growing seedlings; thus, the rate of Cd uptake by the roots may have been limited by the supply of Cd to the root surface, rather than by the concentration of the metal in the rooting solution. This scenario may represent many soil solutions; in such situations, these data suggest that total metal concentration is a better predictor of uptake than is the free-metal ion concentration.

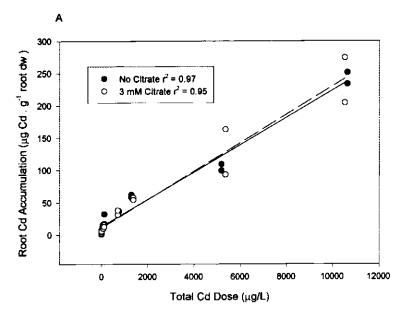
# Capacity of Plants to Take Up Metals from the Environment

Plants take up metals both actively and passively. The surfaces of root cells are loaded with negatively charged sites which weakly bind cations, whether the plant is dead or alive. The purpose of these sites, which occur in the "Donan free space," is to concentrate elements close to the membrane transporters, thus enhancing the uptake rate of essential cations. Since the binding of these sites is non-specific, the same mechanism will also concentrate non-essential cations. In general, roots of monocots such as grasses (Graminae) have half the cation exchange

capacity (CEC) capacity that roots of dicotyledonous species such as legumes exhibit. In addition to this apoplastic binding, metals also cross membranes into the symplastic ("living") system of the plant (Marschner, 1995). The capacity of a plant to take up metals varies widely, even within species. Durum wheat cultivars differ greatly in their capacity to concentrate Cd in grain and are the focus of much interest in the context of food safety.

The question of how cultivars of durum wheat accumulate distinct amounts of grain-Cd from the same soil is

a challenging one, as it involves aspects of genetics, biochemistry, plant physiology and plant morphology (Cieśliński et al., 1996). Previous work in our laboratory examining the accumulation of Cd in roots and shoots of the durum wheat cultivars "Kyle" and "Arcola" over their lifetime demonstrated that "Kyle" (high grain-Cd accumulator) had a lower concentration of Cd in root tissues than "Arcola" (lower grain-Cd accumulator) (Chan, 1996). Using 106 Cd as a tracer isotope, we then focussed on relative uptake and translocation at various points in the growth cycle of the cultivars.



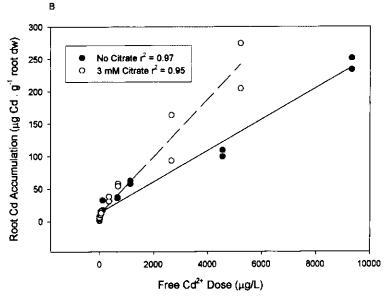


Figure 1 Accumulation of Cd in young durum wheat roots (cv. Arcola) as a function of total element concentration (A) and free ion concentration (B), with and without citrate in the rooting solution.

Results from these experiments showed that the high grain-Cd accumulating cultivar ("Kyle") was taking up Cd and translocating it to flowering heads later in the developmental cycle, whereas the lower grain-Cd accumulating cultivar ("Arcola") stopped translocating Cd from the roots to the shoots before the flowering stage (Chan and Hale, 1998) (Fig. 2). These plants were already well equilibrated to Cd, and so concentration gradients of Cd did not play a role in the uptake or translocation of 106Cd. Our observation that the higher grain-Cd accumulating cultivar was translocating Cd from roots to shoots later into its development than the lower grain-Cd accumulator suggests that internal partitioning mechanisms may be key to

differentiating these cultivars.

We have also examined the role of root morphology in Cd accumulation, using seedlings of these same two cultivars (Berkelaar and Hale, 2000). Studies in other laboratories, using microelectrodes, have indicated that root tips were the most active region of the root in terms of Cd uptake (Piñeros et al., 1998). With this knowledge in mind, we characterized the roots of "Kyle" and "Arcola" in terms of root tips and root surface area, to determine if these morphological characteristics were consistent with the differences in cultivar Cd accumulation. Indeed, "Arcola" had more root tips and greater root surface area than "Kyle," both of which were consistent with its greater accumulation of Cd than "Kyle." To

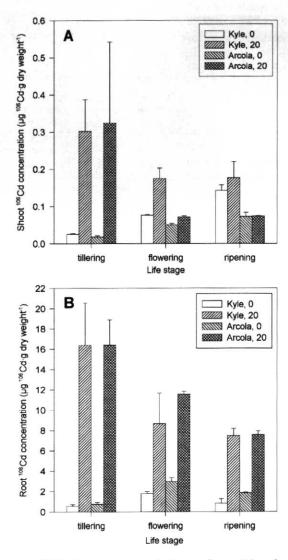


Figure 2 Accumulation of  $^{106}$ Cd from rooting solution in shoots (A) and roots (B) of durum wheat (cvs. Kyle and Arcola) 24 hours after addition of 0 or 20  $\mu$ g·l<sup>-1</sup>  $^{106}$ Cd at three stages of plant development (n=2).

separate the effect of morphology from physiology, Cd content of roots was normalized for the morphological features known to play a role in metal harvesting. Whether expressed on the basis of dry weight, area or number of root tips, "Arcola" had more Cd in the roots than "Kyle" (Table 1), suggesting that internal physiological processes were more important to the accumulation of Cd than morphological characteristics.

# ABSORPTION OF PLANT-INCORPORATED METALS BY MAMMALS

The effect of digestive processes on the chemistry and bioavailability of plantincorporated Cd to mammals is sparingly characterized (Lind et al., 1998), despite the fact that this is the primary Cd exposure pathway for non-smoking humans. Within organisms, cellular processing of the metal after uptake influences the concentration that reaches the metabolic sites of action: the true "bioavailable dose." Cadmium in plants is usually bound to large organic complexes that may differ markedly in their occurrence, binding strengths, sites of activity, or sites of storage, etc. These complexes [phytochelatins (multiple glu-cys pairs), phytates (myo-inositol hexaphosphates) and enzymes] are less water soluble than inorganic Cd salts; however, the enzymes and acids of the mammalian digestive processes may solubilize these complexes before absorption by the intestine.

For risk assessment of metals in foods, it is useful to know whether the absorption of metals from foods is different when the metals are incorporated into the foods versus added to the foods as soluble salts. Testing this hypothesis would provide insight into the importance of metal speciation to digestive absorption, as well as demonstrate whether simple salt amended studies of dietary availability are relevant to risk assessment. We conducted a study of Cd accumulation in the liver and kidney tissue of mice as a result of dietary Cd delivered by one of three ways: Cd was incorporated into the grain during growth; Cd was added to the grain as a soluble salt before consumption; and the soluble salt of Cd was introduced into the animals' stomachs by a tube (gavage) (Chan et al., 2000). There was no

Table 1 Root tissue Cd concentrations for durum wheat (cultivars Kyle and Arcola).			
Unit	Kyle	Arcola	% (Arcola>Kyle)
μg Cd · g <sup>-1</sup> dry weight μg Cd · cm <sup>-2</sup> root area	8	13	63
µg Cd · cm-2 root area	8	11	38
μg Cd · 1000-1 root tips	3.5	5.1	46

difference between plant-incorporated Cd and added soluble Cd salt diets in terms of Cd accumulation in kidney and liver tissues, but accumulation from both of those diets was lower than occurred in gavage diets. These data suggest that digestive processes may render differences in solubility (or bioavailability) among Cd species at ingestion independent of the processes of intestinal absorption.

A companion study was conducted using lettuce diets with incorporated or soluble salt-amended cadmium, fed to rabbits. There was a relationship between the Cd dose, administered using all three methods of delivery, and the accumulation in the kidney; as Cd dose increased, so did the tissue concentration in this organ (Fig. 3). Our data suggest that the bioavailability of Cd that has been incorporated into lettuce was slightly less than for Cd that has been added to the same diet as a soluble salt (Fig. 3). This difference was observed only for accumulation in rabbit kidney and not for the liver. This suggests that estimating absorption of Cd from lettuce using soluble Cd salt-amended diets may slightly overestimate the exposure to the kidney, but not so for the liver. Thus, the use of soluble Cd salt-amended diets for

the purposes of risk assessment of dietary Cd may be appropriate, as the bioavailability of Cd in foods may be not very dependent on Cd speciation in food.

### CONCLUSIONS

The goal of the research described above was to gain some insight into the role of plants in the transfer of cationic metals, particularly Cd, from soils to ecological targets - either themselves or their consumers — and to gain some insight into the importance of "bioavailability" in these transfer processes. It is clear from our work, as well as other studies, that the free ion activity or concentration of Cd in solution is not a good predictor of bioavailability for plants, likely for the reason that one or more of the assumptions associated with the Free Ion Activity Model of Bioavailability will not be met in most terrestrial ecological situations. Further, plant characteristics are modifiers of Cd accumulation, so measurement of bioavailable Cd in soils to broadly predict transfer to plants is not a simple goal. The speciation of Cd in foods appears to have a relatively minor influence on Cd transfer to mammalian consumers, likely because digestive processes reduce a variety of Cd species at ingestion to a

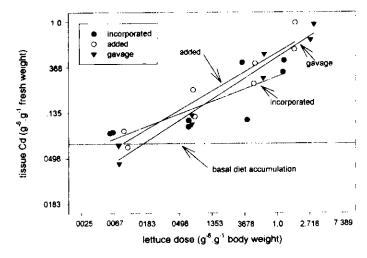


Figure 3 Accumulation of Cd in rabbit kidney relative to ingested dose of Cd, added or incorporated into lettuce diets, or administered by gavage.

more homogenous group, post-digestion. In summary, it seems clear that establishing soil metal concentrations to protect biota require careful consideration of the effects of plants on modifying the apparent concentration of metals that the biota receive from soil.

### **ACKNOWLEDGMENTS**

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