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Quantification of Metal Bioavailability for Lettuce (*Lactuca sativa* L.) in Field Soils

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Abstract. Understanding metal bioavailability of plants in soils requires, apart from physiological processes and symbiosis with arbuscular mycorrhizal fungi, the consideration of the chemical availability in the soil solution (the intensity of the toxic exposure) and the soil's capacity to supply the metal (capacity). In this contribution we report on the time-dependent accumulation of As, Cd, Cr, Cu, Ni, Pb, and Zn in lettuce (*Lactuca sativa* L.). Bioassays with 17 Dutch field soils and two artificially metal-contaminated soils were carried out. Phytotoxicity was observed in soils with pH (pore water) <4.8. Metal uptake is shown to be both metal- and soil-dependent and strongly depends on the amount of water the plant transpired and the available concentration in the water. No net accumulation of As, Pb, Ni, and especially Cr was observed in most soils tested. The latter observation is in agreement with findings of Zayed *et al*. (*Planta*, 1998 206:293–299), who reported that translocation of Cr from roots to shoots is extremely limited. Internal Cd levels in the plants varied greatly among soils, whereas plant tissue concentrations of Zn and especially Cu appear to be regulated at more or less fixed levels. The 0.01 M $CaCl_2$-extractable metal pool provides the best descriptor for the capacity of the soil to supply Cd and Zn. This enabled the development of models that are suited to predict Zn and Cd uptake by lettuce in both field soils (weathered soils) and soils to which metal salts were added, which is common practice in toxicity testing of chemicals. It is concluded that of all metals included in this study, Cd is the metal of most concern due to bioaccumulation through the soil-plant-animal food chain as Cd is the only metal that might pose human or animal health risks at plant tissue concentrations that are not directly phytotoxic. Finally, application of the models for risk assessment purposes is discussed.

There is increasing awareness that chemical criteria that are based on total concentrations in soil are not directly associated to effects of chemicals. Accurate risk assessment of contaminated soils must couple available contaminant concentrations with observed effects data. In general, such coupling should preferably be based on field observations. Often, however, the interpretation of such data is problematic due to a variety of reasons. Among others, it is usually difficult to deduce causal relationships, and variation in the dataset that exists as a consequence of the many external field-related factors is often difficult to qualify, let alone quantify. Therefore, bioassays are often used instead to mimic exposure and induce effects, in general using standardized laboratory conditions in which the impact of external factors, like temperature and humidity, is minimized.

Problems in the use of bioassays usually arise with regard to the extrapolation of laboratory data to realistic field circumstances. This is associated with differences in exposure and actual uptake between laboratory and field settings. It is the issue of bioavailability that plays a key role in this respect. As indicated by Posthuma *et al*. (1998), "bioavailability" should be handled as a dynamic process, comprising at least two distinct and different phases: a physicochemically driven desorption process and a physiologically driven uptake process. The first aspect was defined as "environmental availability," and the second as "environmental bioavailability." The latter requires the identification of specific biotic species as endpoint. It is usually assumed that uptake by soil-inhabiting animals is governed mainly by transport via pore water (see, for instance, Spurgeon and Hopkin 1996). Due to their complex physicochemical behavior, evidence of pore water–related uptake is at present only circumstantial for metals.

Soil organisms potentially have different uptake routes, and the predominance of each route for a species probably depends primarily on its building plan, as reflected in the morphology of the organisms. Weak-bodied animal species for instance generally show a high proportion of uptake through skin transport, whereas hard-bodied species are predominantly exposed via uptake through the gut wall.

Peijnenburg *et al*. (1997) provided guidance to necessary components of risk assessment procedures that take bioavailability explicitly into account. Among others, a framework was presented that, because the issue of availability is explicitly addressed, should yield validated procedures that are predictive of effects in systems that have not been biologically tested. Various uptake routes in different soil biota are to be taken into account in the concept of bioavailability. It is necessary to

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quantify the relevant uptake mechanisms and kinetics of different species and to assess whether a limited number of generally valid uptake and internal transport processes can be associated to various groups of chemical compounds. Eventually, it is mostly the concentration at the target site that counts, as this is directly related with (organ-)effect levels.

Earlier work from this laboratory focused on quantifying environmental availability and bioavailability of metals for two oligochaete species (*Enchytraeus crypticus* [Peijnenburg *et al*. 1999a] and *Eisenia andrei* [Janssen *et al*. 1997; Peijnenburg *et al*. 1999b]). It was found that these soft-bodied invertebrate species are exposed either directly via the pore water or via an exposure route that is linked to the pore water. To investigate whether these findings can be extended to a variety of diverging species, the current work focused on a nonmobile group of species with a different building plan: plants. Here, the results of a dynamic study on metal uptake by lettuce (*Lactuca sativa* L.) are reported. Lettuce is one of the biological species recommended for soil toxicity testing by the OECD (1984). It accumulates metals at relatively high internal levels, given its efficiency of root uptake and subsequent translocation within the roots and the shoots, whereas it is stated by Garate *et al*. (1993) that modulation of the rhizosphere by lettuce is limited. These authors, however, did not take interactions with arbuscular mycorrizal fungi into account. As illustrated by Hildebrandt *et al*. (1999), this symbiosis is of importance for nutrient transfer to the plants, though it takes about 3 weeks before it is fully effective. Plant elemental uptake is, apart from the plants' uptake systems and species specific (cultivar, ecotype) demands, controlled by chemical availability in the soil solution as well as the capacity of the soil to supply that element (Laurie and Manthey 1994). Most plant tissues will accumulate many times the amount of metal available in the soil solution at any given moment. Effectively, the soil solution is "emptied" and replenished many times within even a single day (Bouldin 1989). So plant metal uptake in this case is not only dependent on the environmental availability of the metal in the soil solution (*intensity*) and the particularities of that plant's uptake mechanisms but also on the soils' capacity to supply that particular element (*capacity*), which includes the kinetics of metal supply. In addition, when studying uptake of elements by plants it should be taken into account that it is necessary to distinguish between essential and nonessential elements. Specific ion-uptake systems with high and low affinity for essential elements as well as feedback mechanisms related to the need of the plants for essential elements, have recently been reviewed by Guerinot (2000). In the study reported here, the aspects of supply and demand and physiological feed back mechanisms were indirectly taken into account.

Objectives

The main objectives of the study reported here were to expand the database on metal uptake in field soils by species assumed to be exposed via the pore water or a related exposure route and to quantify the modulation of metal uptake as a function of the relevant metal pools and soil characteristics. Lettuce was selected because its uptake characteristics differ considerably from the animal species tested before. Important in this respect is the decrease of internal metal concentrations that takes place as a consequence of growth of the juvenile plants, in contrast to the limited growth of adult oligochaetes used in the studies mentioned above.

To establish the main objectives of this study, the relationships between accumulated plant tissue concentrations of As, Cd, Cr, Cu, Ni, Pb, and Zn and the soil metal pools was assessed, taking the impact of the most relevant soil and pore water characteristics (like pH, DOC, OM, etc.) into account. Metal pools considered were soil total metal content (HNO_3 digestion), pore water metal content, the soluble metal pool ($CaCl_2$ extraction), and the calculated free metal activity in the pore water. Uptake experiments were carried out with a set of 19 well-characterized metal-contaminated Dutch field soils. Two soils were included in this test set that had artificially elevated metal levels, to study the impact of metal added to the soil (as is commonly done in toxicity testing) on metal bioavailability to lettuce. Several extraction techniques are available for the purpose of simulating the soluble metal pool, like mild neutral salt extractions, extractions with complexing agents, etc. It turned out that results of most mild extractions across a wide range of soils are correlated, so only one mild extraction method was selected in this study. A correlation among mild extractions can also be deduced from an extensive evaluation of extraction media, as carried out by Gupta and Aten (1993). Dry weight of the above-ground tissues of the plants and the metal concentrations in these tissues were determined in the laboratory at preset time intervals.

Although there is a wealth of data on metal uptake by lettuce, to our knowledge the impact of the soil and/or pore water composition on metal bioavailability to lettuce has never been systematically investigated across a wide range of field soils with varying composition. It should be noted that, as indicated above, several plant-related factors may bias internal levels of the metals studied. These factors include (among others) interactions with arbuscular mycorrhizal fungi, specific ion uptake systems for essential metals, additional stress related to the transfer of the plants from a nutrient solution to soils with clearly differing soil composition, and metal gradients between young and old leaves (McKenna *et al*. 1993). Given the main aim of quantifying the modulation of metal uptake by lettuce (*i.e*., quantifying the chemical demand of specific metals by lettuce), these factors were either indirectly taken into account (like interactions with arbuscular mycorrhizal fungi and additional stress due to transfer) or considered to be constant (like the existence of metal gradients between leaves of different age and the presence of specific ion uptake systems).

Materials and Methods

Soil Selection

The 19 Dutch field soils used in this study constitute a subset of a set of 49 soils collected at both moderately contaminated sites, and sites expected to contain metals at background levels (De Groot *et al*. 1998). The set of 49 soils contains a wide range of soil properties, characteristic for The Netherlands. Principal component analysis (PCA) was used to select a subset of soils that is representative for the whole dataset. Geladi and Kowalski (1986) provide details of the PCA method. Seventeen soils were selected with the aim of spanning as far

as possible the space covered by the two principal components in a PCA score plot. Two additional soils, one of which was OECD artificial soil medium (OECD 1984), were selected to study the impact of addition of a mixture of metal salts to the soil (as is commonly done in toxicity testing) on metal bioavailability to *L. sativa* L.

Soil and Pore Water Treatment

Details on soil codes, sample sites, soil collection, soil treatment, chemical analyses, soil characterization, pore water collection, metal concentrations, and chemical speciation calculations are given in De Groot *et al*. (1998). The soil characteristics as well as metal contamination levels differ strongly, and variability among soils was large for the most relevant factors influencing metal partitioning and uptake, as was aimed at in the sampling procedure. OECD artificial soil medium (soil code X) was prepared according to the OECD protocol (OECD 1984). An aqueous solution of a mixture of metal salts was added to this soil and to the soil encoded AQ. Sample AR was taken at the same site as soil AQ, but no metals were added to sample AR. The soils X and AQ were allowed to equilibrate for at least 3 months at 4°C. $CaCl_2$ extraction was carried out for a limited number of soils following 35-day exposure of the plants.

Cultivation and Exposure of Plants

All experiments were carried out in a climate room with constant temperature (20 ± 3°C), a day/night regime of 16/8 h and a light intensity of 3,000 lux. Relative humidity was about 65%. Plants (*L. sativa* var. *longifolia*) were cultivated for about 14 days in a Steiner nutrient solution (Steiner 1961), using perlite as inert support medium. Plastic jars (250 ml) were filled with about 250 g of soil set at pF 2 humidity by addition of 0.002 M $Ca(NO_3)_2$, and acclimated for at least 24 h. After 14 days of cultivation, three randomly selected plants were transferred to each jar. The weight of the jars was kept constant by daily addition of distilled water. After 1, 3, 7, 10, 14, 21, 28, and 35 days of exposure, all shoot material within one jar (*i.e*., three plants) was harvested, rinsed with demineralized water, blotted between paper towels, weighed (wet weight), dried at 70°C for at least 24 h, weighed (dry weight), ground, and used for analyses of metal contents. A similar procedure was carried out for plants that were not exposed to any of the soils, and hence were sampled directly from the nutrient solution ($t = 0$).

Digestions and Analyses of Plant Material

Dried plant tissue (three plants) was weighed into destruction tubes. Five milliliter of MilliQ water, 10 ml concentrated nitric acid, and 0.2 ml concentrated HF were added to each tube. The plants were destroyed in a microwave oven for 60 min at a constant temperature of 185°C. The destruate was quantitatively transferred into a 50-ml volumetric flask. Metal concentrations in the digests were measured for As, Cd, Cr, Ni, and Pb with graphite furnace AAS (Perkin Elmer-5100 PC with Zeeman background correction). Zn and Cu were analyzed by flame AAS (AAnalyst Perkin Elmer with deuterium background correction). Metal concentrations in the plant tissue were expressed on a mmol · kg^{-1} dry weight basis. Blanks and reference materials (tomato leaves [U.S. Department of Commerce, National Institute of Standards and Technology, Gaithersburg, MD, reference material 1573a], and Virginia tobacco leaves [Polish Academy of Sciences and Institute of Nuclear Chemistry and Technology, Warsaw, Poland, code: CTA-VTL-2]) were digested simultaneously.

Data Analysis

Modeling Growth and Metal Uptake. Plants grew considerably during the experiments. Growth and water-driven uptake were modeled following current methodologies, in combination yielding a parameter that quantifies true uptake (k_x) as follows. First, growth was described by means of a growth model that fitted well to the data collected. Second, uptake was envisaged as a passive process related to the amount of water transpired. Linking internal metal levels in the plants to the growth model yielded k_x, a characteristic parameter for uptake.

To describe the growth of the plants, the Von Bertalanffy growth model was applied, from Sneller *et al*. (1999). Although this model is usually selected to describe growth in animals, it provided a satisfactory fit to the current data. The model is based on the consideration that resources for growth are the difference between the uptake of resources (related to the organism's surface area) and the costs for maintenance (related to the organism's volume):

$$\frac{dV(t)}{dt} = aV^{2/3}(t) - bV(t) \tag{1}$$

In this equation, V is the organism's volume (m^3) and a and b are the parameters related to uptake and maintenance, respectively (m · day^{-1} and day^{-1}). The general solution to this model is:

$$V(t) = \left[\frac{a}{b} - \left(\frac{a}{b} - V_0^{1/3}\right) \cdot e^{-1/3bt}\right]^3 \tag{2}$$

This leads to an S-shaped curve with a maximum volume:

$$V_\infty = \left(\frac{a}{b}\right)^3 \tag{3}$$

When there is a constant relationship between volume and weight (*i.e*., a constant bulk density), the same model can be applied to describe the change of the organism's weight in time.

For neutral organic xenobiotics, it is generally assumed that plant uptake is a passive process, leading to a model formulation where uptake is related to the chemical's concentration in pore water and the transpiration stream of the plant (Trapp and Matthies 1995). The removal processes in the plant include volatilization from the leaves, metabolism, and growth (not a true removal process, but the effect is similar on a concentration basis). This description of accumulation is essentially different from the uptake in, *e.g*., earthworms, where a concentration gradient drives uptake. Naturally, the accumulation of metals is modified by far more factors, for instance, regulation, membrane properties, root exudates, and incorporation into organic molecules. However, for the modeling procedure and as zero hypothesis we will assume that passive uptake with the transpiration stream is the dominant process. If metals are taken up passively by the plants, the rate of uptake only depends on the amount of water the plant transpires and the available concentration in the water. Growth will be the only removal process as volatilization and metabolism are not relevant for the metals of interest.

When external conditions like temperature and humidity are kept constant (as in this study), the amount of water transpired is related to the growth of the plant. The plant needs to open its stomata to obtain CO_2 from the air, but at the same time water is lost through this pathway. A minor amount of water is lost through the cuticles, independent of the stomata. When the transpiration stream is responsible for the accumulation, the uptake flux of the metal is linearly related to the growth rate. In other words, for each kg of dry matter increase, a certain amount of CO_2 is required, whereby a certain amount of water is transpired (the so-called water use efficiency, WUE), leading to uptake of a certain amount of metal. The WUE differs between plants but is approximately 800 L · kg^{-1}DW for green vegetables (Flindt

1988) (although this value may be influenced by factors such as nutrition, stress, and CO_2 levels). In equation form this leads to:

$$\frac{dA(t)}{dt} = C_w \cdot WUE \cdot F_t \cdot \frac{dW(t)}{dt} \quad (4)$$

with A = amount of metal in plant (mol), W = weight of plant (kg DW), t = time (days), C_w = concentration in water (mol · L^{-1}), WUE = amount of transpiration per kg DW produced (L · kg^{-1} DW], F_t = concentration ratio between pore water and xylem (−).

As we assumed a constant bulk density and since the parameters C_w, WUE, and F_t are assumed constant, the amount of metal in the plant follows a Von Bertalanffy curve similar to the volume of the plant. The shape will be similar to the growth curve but the three "constants" will modify its position. Fitting Equation 2 (in its log-transformed mode) to the log-transformed growth data (dry weights of the plant tissues) yielded the characteristic values of a (g · day^{-1}), b (day^{-1}), and V_0 (g), which were used as constants to fit the following model to the experimentally determined amount of metal in the plant:

$$\mathrm{Log}(A(t)) = \mathrm{Log}\left(k_x \cdot \left[\frac{a}{b} - \left(\frac{a}{b} - V_0^{1/3} \right) e^{-1/3bt} \right]^3 - k_x \cdot V_0 + A_0 \right) \quad (5)$$

A_0 is the total amount of metal present in the plant at the start of the uptake experiment, V_0 represents the weight of the plants at the start of the experiment, and k_x represents the amount of metal taken up by the plant per kg of DW produced (mol · kg^{-1} DW). k_x is the (soil- and pore water–dependent) parameter that is characteristic for the link between actual metal uptake and metal bioavailability among soils, and it is the parameter used for further modeling. Provided that the amount of metal present in the plant at the start of the experiment (A_0) is small as compared to the amount of metal present at equilibrium ($k_x \cdot V_\infty$), k_x may be considered representative for the steady-state metal concentration in the plant.

Relating Uptake to Soil and Pore Water Characteristics. Empirical formulas were derived to investigate whether the variation expected in k_x values for the test set of 19 soils was statistically associated to the influence of metal pools, soil characteristics, or both. Stepwise regression was used to identify the crucial characteristics explaining most of the variation in k_x. The impact of the most relevant soil and pore water characteristics (like pH, DOC, OM, etc.) was explicitly taken into account in the regression procedures. The multivariate functions take the form:

$$\mathrm{Log}(k_x) = a\ \mathrm{Log}(A) + b\ \mathrm{Log}(B) + \ldots c \quad (6)$$

with A and B = descriptors, *i.e.*, different soil and pore water characteristics and the soil and pore water metal pools; and a, b, and c = coefficients. The significant descriptors are arranged in decreasing order of importance (A, B, and so forth). Descriptors that do not explain a significant part of the variation ($p > 0.05$) are not incorporated in the formulas. Metal pools considered were soil total metal content (HNO_3 digestion), pore water metal content, the soluble metal pool ($CaCl_2$ extraction), and the calculated free metal activity in the pore water. Note that statistical association does not necessarily imply a mechanistic explanation.

Results

Selection and Characterization of Soils

The scores of the soils for the first two principal components are shown in Figure 1. Some clustering of the soil samples can be distinguished. The soils are distributed over the score plot, which is a reflection of variation of the soil characteristics among sites according to the criteria used for the selection of the sampling sites. The soils U and AH do not fall within the 95% confidence interval. This is due to their high organic matter content. A subset of 17 soils was selected with the aim of spanning as far as possible the space covered by the two principal components in the score plot. Soils X (OECD artificial medium) and AQ were added to this set to reach a total of 19 soils.

The metal concentrations in the soil solid phase, the pore water, and the 0.01 M $CaCl_2$ extracts, as well as the pH of the pore water are given in Table 1. Additional information on the composition of the soils can be found in De Groot *et al*. (1998). When interpreting the relationships between metal levels in plant tissue and external metal concentrations, it should be noted that pore water concentrations of Cd and Zn are correlated to Cd and Zn levels in the $CaCl_2$ extracts. The correlations are given in Table 2. No significant correlations were found for the other metals studied.

Bioassays—General Observations

Abnormalities with regard to growth of the plants and apparent adverse effects of soil characteristics on lettuce were observed in 3 out of the 19 soils tested. Plants grown in soil M turned yellow in the first few days of cultivation and then slowly wilted. Similar observations were made for soil AC (actually, decreasing dry weights of the shoot material was observed), whereas plants cultivated in soil AE died within the first few days of exposure. Inspection of Table 1 suggests that the pH of the pore water may be used to discriminate between soils in which adverse effects on lettuce are observable, and soils for which this is not the case. Tentatively, a critical pH value of about 4.7 can be set for this purpose. As bioavailability can only be quantified among soils if plants do equally well, soils M, AC, and AE were classified as being not useful for quantifying metal uptake and hence were not included in further data analyses.

Table 3 shows the results of fitting Equation 2 (in its log-transformed form) to the growth data obtained. In general, the model could be fitted quite well to the data, with values of $R^2_{adj} > 0.84$ for all soils. A typical growth curve is given in Figure 2.

Bioassays—Metal Uptake Characteristics

Detailed information on internal metal levels in plants exposed in the soils employed in this study is available on request. As an illustration of typical uptake patterns observed, the time-dependent accumulation of As, Cd, Cu, and Ni (no accumulation) in soil S is given in Figure 3. The values of a, b, and V_0 given in Table 3 were used as constants for fitting the modified Von Bertalanffy model (Equation 5) to the total amount of metal accumulated in the plant tissue over time. The resulting values of k_x are given in Table 4 for each of the metals considered. Again, the model could be fitted quite well to the data.

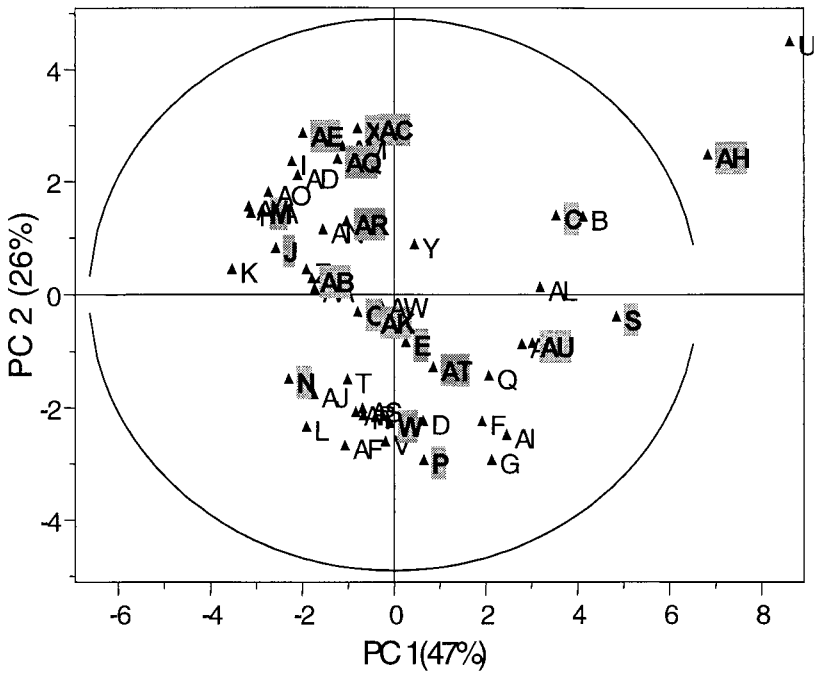

Fig. 1. Principal component (PC) score plot of the soils sampled. The ellipsoid indicates the 95% confidence interval. See De Groot *et al.* (1998) for soil codes and soil characteristics. The codes marked in bold represent the subset of 19 soils selected in this study

In most soils tested, there was no accumulation of Ni and especially Cr. Due to growth dilution, plant tissue concentrations of Cr and Ni in most soils decreased with increasing exposure times. The total amount of metal in the plant, however, remained constant. The sole exceptions to this general observation were soils S and AR in case of Cr, and soils AH, AQ, and AR in case of Ni. Significant accumulation of As and Pb was observed in six and eight soils, respectively. It should be noted that rather irregular uptake patterns were found for all four metals mentioned, often including metal levels below the analytical detection limit. This is reflected in the relatively low values of R^2 reported for these metals in Table 4.

Cd, Cu, and Zn were accumulated in the plants in all 16 soils for which no abnormalities were observed. Marked differences among k_x values were found for these three metals: The range of k_x values was greatest for Cd (maximum difference of over 2.7 orders of magnitude), followed by Zn (maximum difference of 1.7 orders of magnitude). Finally, despite the wide range of soil and pore water characteristics of the soils included in the test set, k_x values for Cu are fairly constant among all soils tested (maximum difference of 0.9 orders of magnitude). These findings may well be related to regulation of internal levels of the essential elements Zn and especially Cu in the shoot material.

$CaCl_2$ extraction (0.01 M) was carried out after completion of the uptake experiments to deduce whether metal uptake by the plants might have impacted the easily exchangeable metal pools. One of the reasons for selecting this particular extraction technique was the close relationship that exists between $CaCl_2$-extractable metal levels and pore water concentrations (Zn/Cd: see above). It was found that in general no significant changes were detectable between 0.01 M $CaCl_2$-extractable metal levels at the start and at the end of the experimental period. Also, pH and conductivity of the samples remained constant (data not shown).

Impact of Metal Pools and Soil and Pore Water Characteristics on Metal Uptake

Considering the experimentally determined soil and pore water characteristics and the results of the metal extractions carried out for the soils included in the test, there was no means of discriminating soils in which accumulation of As, Cr, Ni, and Pb did take place from those in which there was no metal uptake by the plants. Therefore it may be deduced from Table 4 that especially for As, Cr, and Ni there is little variance among the experimentally determined k_x values. Given the limited datasets for uptake of As, Cr, Ni, and Pb and given these findings, it was decided to further restrict data analysis to Cd, Cu, and Zn.

As suggested above, active regulation of Zn and especially Cu may have impacted the k_x values found for these metals. As a consequence of active regulation of internal metal levels, the link between the experimental values of k_x as determined in this study and external metal pools or soil and pore water characteristics is expected to be weak. Partly due to the limited variance in the experimental k_x values for Cu, no significant relationship was found between k_x values and any of the metal pools or the soil and pore water characteristics considered.

The range of k_x values found for Zn was intermediate the ranges found for Cu and Cd. As stated above, it cannot be excluded that the k_x values found for the essential element Zn are impacted by regulation. It was nevertheless attempted to correlate experimental k_x values for Zn to external metal pools and a number of soil and pore water characteristics. The relationship between the experimentally determined k_x values in the 16 soils and the soil metal pools was assessed for Cd and Zn as a multivariate function of the measured soil and pore water characteristics. Given the potential dependence of metal uptake by plants on bioavailable metal pools that take the

Table 1. Log-transformed metal concentrations in the soil solid phase (aqua regia), the pore water, and the 0.01 M $CaCl_2$ extracts, and the pH of the pore water for all soils used in this study

		Total Concentration Soil Solid Phase (mmol/kg)							0.01 M $CaCl_2$ Extraction (μmol/kg)							Pore Water (μmol/L)						
Site	pH	As	Cd	Cr	Cu	Ni	Pb	Zn	As	Cd	Cr	Cu	Ni	Pb	Zn	As	Cd	Cr	Cu	Ni	Pb	Zn
C	5.88	−0.694	−2.415	0.303	0.217	−0.082	−0.508	0.283	−1.541	−0.645	−0.737	0.573	0.934	−1.256	1.451	−1.347	−2.066	−0.866	0.494	−0.143	−0.932	0.301
E	6.96	−0.635	−1.140	−0.117	−0.392	−0.351	0.632	1.678	−0.962	0.480	< d.l.	−0.065	0.372	−0.858	2.879	−1.854	−0.815	−1.721	0.137	−0.256	−1.538	1.799
J	5.26	−1.144	−2.066	−0.715	−1.156	−1.510	−0.971	−0.073	−0.923	0.392	< d.l.	−0.720	0.443	−0.312	2.303	−1.796	−0.709	−1.284	0.167	−0.140	−1.638	1.707
M	4.63	−1.469	−3.402	−0.398	−1.049	−0.958	−1.177	−0.543	−1.095	−0.726	−0.769	0.362	0.584	0.110	1.423	−1.668	−1.353	−0.945	0.294	−0.090	−1.190	1.109
N	7.16	−1.008	−2.566	0.137	0.207	−0.351	−0.182	0.484	−1.689	−1.177	< d.l.	0.554	−0.576	−1.213	0.433	−2.301	−2.222	−1.420	0.310	−0.532	−1.377	−0.097
O	6.21	−0.703	−1.271	−0.051	−0.514	−0.768	−0.923	0.358	−1.089	0.579	−0.791	0.073	0.225	< d.l.	1.916	−2.000	−0.573	−1.125	0.307	−0.113	−2.097	1.204
P	7.12	−0.494	−1.425	0.463	−0.074	−0.381	< d.l.	0.950	−0.352	−0.588	−1.144	0.291	−0.197	< d.l.	0.960	−1.301	−1.360	−1.357	−0.009	−0.348	< d.l.	0.732
S	7.17	−0.180	−0.785	0.486	0.329	−0.011	0.262	1.302	−0.687	−0.013	−0.944	0.086	−0.022	< d.l.	1.327	−1.495	−1.650	−1.149	−0.013	−0.561	−1.854	0.041
W	7.50	−0.486	−2.449	−0.086	−0.613	−0.666	−0.778	0.113	0.432	−1.639	< d.l.	−0.180	−0.566	< d.l.	< d.l.	−0.087	−1.527	−1.347	0.134	−0.170	−1.921	0.462
X	5.30	−0.757	−1.226	−0.190	−0.214	−0.405	−0.153	0.902	1.147	1.441	0.924	0.659	2.117	0.578	3.591	1.459	0.983	0.712	0.913	1.644	0.000	3.096
AB	6.04	−1.730	−2.635	−0.457	−0.742	−0.925	−0.774	0.700	−1.409	−0.360	−1.238	−0.318	−0.043	−0.972	2.995	−2.097	−1.836	−1.301	−0.337	−0.752	−1.268	1.831
AC	4.05	−1.676	−2.667	−0.924	−1.471	−1.455	−0.946	−0.797	−1.216	0.117	−1.078	< d.l.	0.279	0.645	1.608	−0.866	−1.575	−0.521	0.117	−0.469	−0.359	0.431
AE	3.49	−1.664	< d.l.	−0.830	−1.678	−1.772	−1.143	−1.011	−0.888	−0.995	−0.923	−0.768	0.071	0.546	1.160	−1.022	−1.370	−1.071	0.396	−0.383	−0.493	0.699
AH	5.35	−0.595	−2.189	0.102	−0.370	−0.259	−0.610	0.168	−2.029	−0.445	−1.038	−0.346	0.612	−1.249	1.601	−1.174	−1.767	−0.529	0.253	−0.021	−0.893	0.362
AK	6.83	−1.157	−1.991	−0.609	−0.271	−0.757	−0.210	1.282	−0.909	−0.465	< d.l.	−0.153	−0.190	< d.l.	2.639	−1.569	−2.114	−1.523	−0.201	−0.724	−1.167	1.212
AQ	4.80	−0.868	−1.557	−0.254	−0.328	−0.626	−0.234	0.593	0.145	1.037	−0.058	0.558	1.692	0.154	3.267	−0.519	0.200	−0.339	0.127	0.958	−0.440	2.551
AR	5.31	−1.278	−2.530	−0.182	−0.670	−0.728	−0.646	−0.163	−0.715	−0.080	−0.695	0.034	0.514	−0.462	2.201	−1.481	−1.295	−1.022	0.316	−0.109	−1.174	1.316
AT	7.40	−1.039	−2.141	−0.325	−0.266	−0.654	−0.326	0.404	−0.288	−1.645	< d.l.	0.086	−0.228	−1.036	0.570	−1.022	−2.319	−1.357	−0.180	−0.592	−1.495	−0.046
AU	7.50	−0.708	−2.368	−0.082	−0.433	−0.561	−0.264	0.350	−0.898	−1.892	< d.l.	−0.226	< d.l.	< d.l.	0.547	−1.387	−2.481	−1.854	−0.155	−0.533	−1.886	−0.699

< d.l. = Below detection limit.

Table 2. Relationship between (log-transformed) pore water concentrations of Cd and Zn and (log-transformed) 0.01 M $CaCl_2$-extractable Cd and Zn contents for the soils included in the test set

$\text{Log } [Zn]_{\text{pore water}} = -0.75 + 0.94 \text{ Log } [Zn]_{CaCl_2}$	$R_{adj}^2 = 0.848$, n = 18, F = 95.5, $p < 0.001$
$\text{Log } [Cd]_{\text{pore water}} = -1.07 + 0.82 \text{ Log } [Cd]_{CaCl_2}$	$R_{adj}^2 = 0.700$, n = 19, F = 43.1, $p < 0.001$

Table 3. Growth characteristics for lettuce plants grown in 19 Dutch field soils

Site	a (g · day^{-1})	b (day^{-1})	V_0 (g)	$(a/b)^3$ (g)	R^2
C	0.08	0.07	0.02	1.31	0.97
E	0.10	0.09	0.04	1.40	0.98
J	0.11	0.14	0.03	0.42	0.94
M	0.16	0.33	0.03	0.11	0.89
N	0.10	0.13	0.03	0.45	0.93
O	0.08	0.05	0.03	3.70	0.98
P	0.09	0.10	0.03	0.80	0.95
S	0.12	0.08	0.03	3.45	0.99
W	0.18	0.16	0.03	1.43	0.99
X	0.07	0.05	0.03	2.78	0.94
AA	0.15	0.09	0.10	4.74	0.98
AC	Decreasing dry weights shoot material				
AE	Plants died within first days of exposure				
AH	0.12	0.06	0.04	10.02	0.95
AK	0.10	0.02	0.02	240.16	1.00
AQ	0.26	0.34	0.04	0.47	0.97
AR	0.15	0.05	0.10	22.85	0.98
AT	0.14	0.05	0.04	21.59	0.98
AU	0.09	0.01	0.05	269.50	0.96

Data were obtained by applying the Von Bertalanffy growth model (Equation 2) to the log-transformed dry weights of the shoot material of plants cultivated during periods of time ranging from 0–35 days.

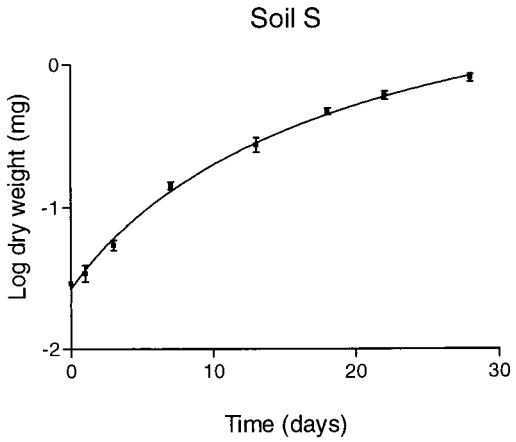

Fig. 2. Typical example of a growth curve for *L. sativa* L., as observed for soil S. The dry weight is the average dry weight of three plants. Bars indicated standard errors

environmental availability (intensity) and the soils' capacity to supply metals into account, the data analyses were at first restricted to developing monovariate relationships between k_x values and the metal pools considered in this study. The results of these analyses are given in Table 5.

As can be deduced from Table 5, most of the variance in the data could be explained on the basis of the simulated metal pools. The highest values of R_{adj}^2 (0.76 and 0.81 for Cd and Zn, respectively) were obtained for the $CaCl_2$-extractable metal pool. Inclusion of additional soil- and pore water–related parameters in general did not significantly enhance the equations found. The sole exception to this general observation was the finding that in case of Zn, addition of the pH of the pore water as an additional descriptor to the total Zn concentration in the soil, significantly improved the monovariate formula with total zinc as the sole descriptor. In Figure 4, k_x values calculated on the basis of the correlations for $CaCl_2$ extraction given in Table 5 are plotted versus experimentally determined k_x values for Cd and Zn.

Discussion

General

The results presented in this contribution show that metal uptake by *L. sativa* L. is metal-dependent. On the one hand, no significant uptake of As, Cr, or Ni is observed. Apparently, either the plant root acts as an effective barrier for these metals or the low solubility and strong retention of these metals in soil prohibit uptake due to low bioavailable concentrations in the soil solution of the soils studied.

Plant tissue concentrations of Cd vary by about 2.7 orders of magnitude and are linked to external metal pools, whereas the plants apparently were capable of regulating their internal Cu concentrations at a fairly constant level. As a consequence, there is no link between internal Cu concentrations and either any of the Cu pools in the soils, or any of the soil and pore water characteristics considered. Sauvé *et al*. (1996) reported Cu tissue concentrations in lettuce after exposure in eight contaminated soils from urban areas. The Cu levels found by Sauvé *et al*. (1996) correspond well with the levels reported in this study. Contrary to Sauvé *et al*. (1996), we were not able to link cupric ion activity to the Cu tissue concentrations found, as is to be expected in case of active Cu regulation by the plants. It should be noted that in the study of Sauvé *et al*. (1996), no plant growth data were given with which to evaluate toxicity of Cu in these soils.

The range of variance of internal Zn concentrations was intermediate the ranges found for Cd and Cu. It cannot be deduced to which extend this finding is related to active regulation of internal Zn tissue concentrations.

Mechanistic Aspects—Applicability of Correlations Found

Although correlations as given in Table 5 do not provide clues for the presence or absence of mechanistic influences of soil or pore water variables on uptake, the findings reported here show that metal uptake of nonessential elements by lettuce is probably best described as a passive process, resulting from the

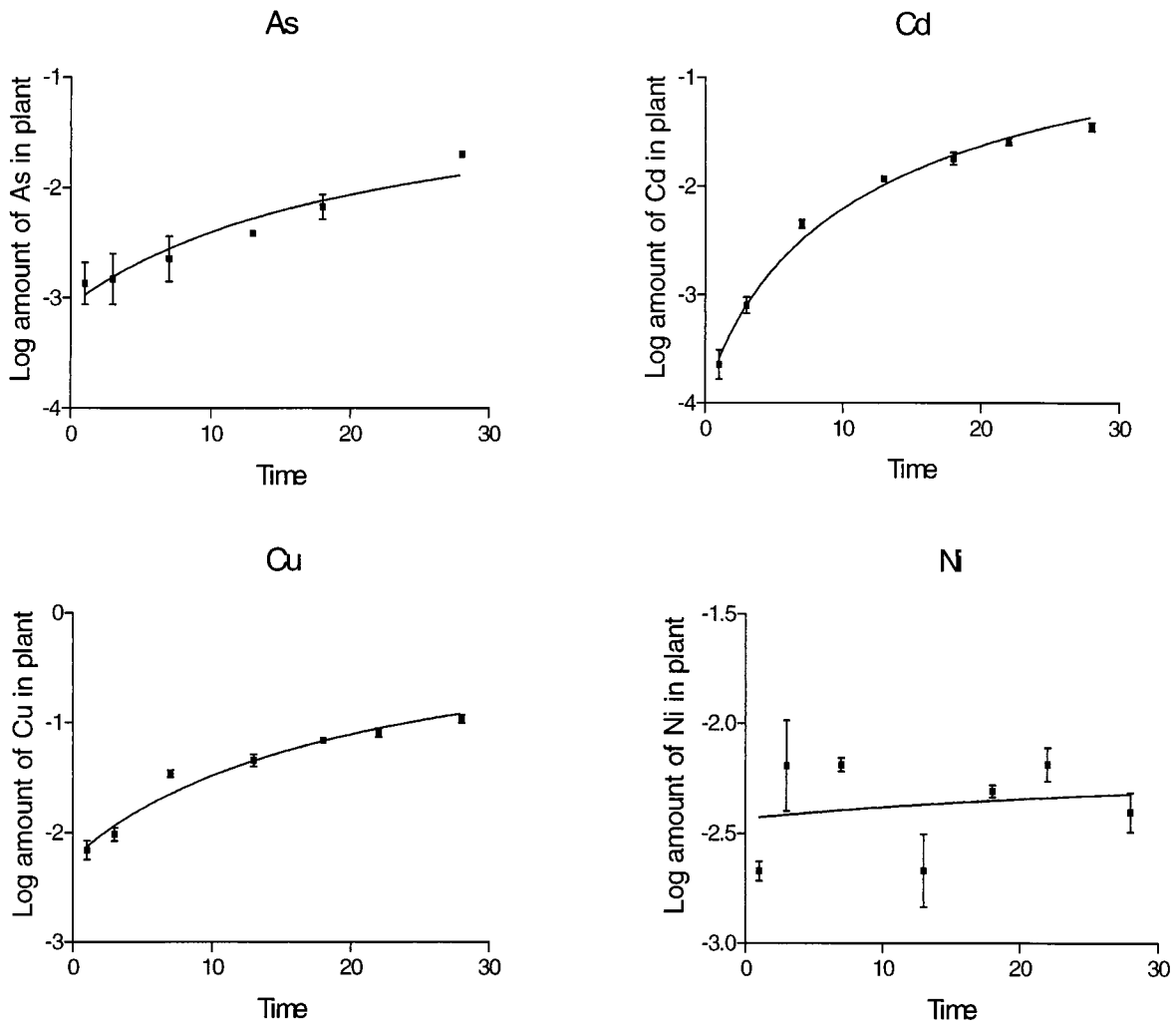

Fig. 3. Time-dependent accumulation of As, Cd, Cu, and Ni (no accumulation) as observed in soil S. The log-transformed amount of metal accumulated (μmol per plant) is given as a function of exposure time (days)

uptake of the pore water needed for growth and transpiration. The fit of the simplistic model to the accumulation data showed that accumulation can be described by the same curve shape as growth. Although the model is crude and only loosely based on plant physiology, the satisfactory fit provided sufficient basis for the multi-variate regressions.

Internal concentrations of essential metals (especially Cu) may well be regulated at fixed levels. As a consequence, growth and transpiration will have a limited effect on levels of regulated metals. This was nicely illustrated by Wilkinson *et al.* (1968), who showed that Zn uptake by wheat plants was unchanged, despite plant transpiration changing almost four-fold between treatments, indicating a close control of Zn absorption rates by the roots.

As we found that plant tissue concentrations of Cd and Zn are related to the soluble metal pools, it may be deduced that the kinetics of replenishment of Cd and Zn taken up from the soil solution are sufficient to maintain fixed pore water concentrations. These findings are supported by the observation that 0.01 M $CaCl_2$-extractable metal concentrations after a maximum of 35 days of exposure are similar to those at the start of the experiments. It should be noted, however, that metal depletion in the rhizosphere is the most important factor to be taken into account in this respect. At present, reliable methods for measuring metal depletion at the plant root/soil interface are lacking. Because only a limited number of mild extraction techniques were employed in this study, it cannot be excluded that additional methods for simulating the soluble metal pool in the rhizosphere would provide an even better relationship with Cd and Zn concentrations in the plant tissues. In view of the similarities observed among mild neutral salt extractions, extraction of the soils with for instance complexing agents (like EDTA/DTPA) might provide a better estimate of this bioavailable metal pool.

Both field soils and artificially contaminated soils (including one artificial soil medium, OECD-soil) constitute the test set. Metal uptake by lettuce exposed in the two soils to which metal salts were added did not deviate from uptake in the field soils (as illustrated in Figure 4). It can therefore be concluded that the regression formulas given in Table 5 are applicable to both field soils (weathered soils) and to soils to which metal salts have been added for a period of at least 3 months prior to

Table 4. Log-transformed values of k_x (μmol · g DW^{-1}), obtained by applying the modified Von Bertalanffy model (Equation 5) to the experimentally determined total amount of metal accumulated in the plant tissue over time

Site	As	R^2	Cd	R^2	Cr	R^2	Cu	R^2	Ni	R^2	Pb	R^2	Zn	R^2
C	n.a.		−1.643	0.968	n.a.		−0.796	0.946	n.a.		n.a.		−0.114	0.961
E	n.a.		−1.226	0.992	n.a.		−0.783	0.820	n.a.		n.a.		0.713	0.988
J	n.a.		−1.259	0.981	n.a.		−0.992	0.598	n.a.		−1.887	0.700	0.648	0.797
N	n.a.		−2.329	0.962	n.a.		−0.640	0.953	n.a.		n.a.		0.058	0.973
O	n.a.		−0.558	0.977	n.a.		−0.835	0.855	n.a.		−1.777	0.850	0.245	0.929
P	n.a.		−1.888	0.932	n.a.		−0.678	0.898	n.a.		−2.048	0.508	−0.118	0.929
S	−1.978	0.719	−1.203	0.986	−1.987	0.529	−0.814	0.951	n.a.		n.a.		−0.189	0.958
W	−1.458	0.973	−2.148	0.965	n.a.		−1.099	0.958	n.a.		n.a.		−0.537	0.969
X	n.a.		−0.643	0.878	n.a.		−1.542	0.837	n.a.		−1.417	0.640	1.217	0.798
AA	−1.891	0.842	−2.341	0.940	n.a.		−0.894	0.935	n.a.		−2.124	0.715	0.693	0.966
AH	n.a.		−1.444	0.989	n.a.		−1.123	0.970	−2.004	0.893	−3.174	0.503	0.162	0.985
AK	n.a.		−2.227	0.940	n.a.		−0.720	0.908	n.a.		−3.082	0.695	0.293	0.971
AQ	−2.123	0.873	−0.988	0.950	n.a.		−1.185	0.656	−1.028	0.942	n.a.		0.989	0.973
AR	−1.747	0.848	−2.011	0.950	−2.152	0.638	−1.103	0.942	−1.956	0.709	n.a.		0.184	0.904
AT	−2.126	0.891	−2.644	0.840	n.a.		−1.201	0.924	n.a.		−2.953	0.521	−0.189	0.963
AU	n.a.		−3.306	0.856	n.a.		−1.351	0.956	n.a.		n.a.		−0.161	0.947
Minimum	−2.126		−3.306		−2.152		−1.542		−2.004		−3.174		−0.537	
Maximum	−1.458		−0.558		−1.987		−0.640		−1.028		−1.417		1.217	
Average	−1.887		−1.741		−2.070		−0.985		−1.663		−2.308		0.243	

The value of the regression coefficient, R^2, is given as an indication of the goodness of fit. Only soils in which no abnormalities with regard to growth of the plants and apparent toxic effects were observed are included in this table (n.a. = not available: metal levels below detection limit).

Table 5. Mono- and multivariate regression formulae relating (log-transformed) k_x values for accumulation of Cd and Zn in *L. sativa* L. after exposure in 16 Dutch soils to (log-transformed) external metal pools and soil characteristics

Metal	Metal Pool	Equation Obtained	Statistics
Cd	Total Cd concentration in soil (mmol/kg)	Log $k_x = -1.73 + 0.02\ \mathrm{Log[Cd]_{soil}}$	$R^2_{adj} = -0.07$, n = 16, SE = 0.78, F = 0.002, p = 0.96
	0.01 M $CaCl_2$-extractable Cd (μmol/kg)	Log $k_x = -1.52 + 0.69\ \mathrm{Log[Cd]_{CaCl_2}}$	$R^2_{adj} = 0.76$, n = 16, SE = 0.37, F = 49.7, p < 0.001
	Total Cd in pore water (μmol/L)	Log $k_x = -0.88 + 0.64\ \mathrm{Log[Cd]_{pw}}$	$R^2_{adj} = 0.63$, n = 16, SE = 0.46, F = 26.5, p < 0.001
	Cd activity in pore water (mol/L)	Log $k_x = 2.87 + 0.59\ \mathrm{Log\ (Cd)_{act}}$	$R^2_{adj} = 0.56$, n = 15, SE = 0.42, F = 18.5, p = 0.001
Zn	Total Zn concentration in soil (mmol/kg)	Log $k_x = 0.11 + 0.24\ \mathrm{Log[Zn]_{soil}}$	$R^2_{adj} = -0.004$, n = 16, SE = 0.49, F = 0.94, p = 0.349
	0.01 M $CaCl_2$-extractable Zn (μmol/kg)	Log $k_x = -0.47 + 0.40\ \mathrm{Log\ [Zn]_{CaCl_2}}$	$R^2_{adj} = 0.81$, n = 15, SE = 0.20, F = 59.7, p < 0.001
	Total Zn in pore water (μmol/L)	Log $k_x = -0.17 + 0.42\ \mathrm{Log[Zn]_{pw}}$	$R^2_{adj} = 0.79$, n = 16, SE = 0.22, F = 58.6, p < 0.001
	Zn activity in pore water (mol/L)	Log $k_x = 2.70 + 0.46\ \mathrm{Log(Zn)_{act}}$	$R^2_{adj} = 0.78$, n = 16, SE = 0.23, F = 54.3, p < 0.001
	pH (pw)	Log $k_x = 2.50 - 0.35$ pH	$R^2_{adj} = 0.43$, n = 16, SE = 0.37, F = 12.6, p = 0.003
	Total Zn in soil (mmol/kg) + pH (pw)	Log $k_x = 2.80 + 0.50\ \mathrm{Log(Zn)_{soil}} - 0.45$ pH	$R^2_{adj} = 0.70$, n = 16, SE = 0.27, F = 18.2, p = 0.001

exposure of the plants. The latter is common procedure in toxicity testing and hence in these cases too, $CaCl_2$ extraction provides a good means of estimating environmental metal bioavailability to *L. sativa* L.

Toxicity

Toxic effects caused by any of the soil characteristics, including metals and organic toxicants present in the soils, as well as factors such as pH, may directly affect plant behavior and metal accumulation and thus may bias the findings. Both metal uptake and growth may be affected. In addition, factors like nutrient availability and even the amount of soil used per plant may have impacted the growth characteristics observed in this study, and hence metal uptake. Moreover, possible effects due to the presence of excess amounts of organic toxicants cannot be completely ruled out because levels of a broad range of organic substances were not monitored in the soils tested. However, soils in which there were clear effects on plant performance were excluded from the analyses. Toxic effects in the remaining soils may still be present, as judged using literature data. The following critical NOEC values for lettuce (tissue concentrations on a dry weight basis) are given by

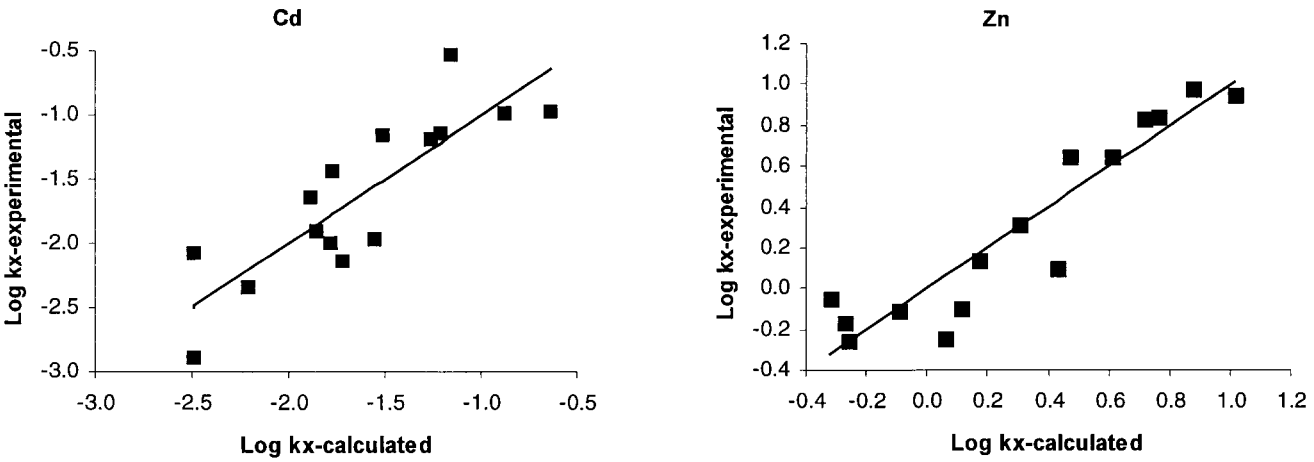

Fig. 4. Comparison of experimental and predicted log-transformed k_x values (μmol · g DW^{-1}), on the basis of the $CaCl_2$-extractable Cd and Zn pools

MacNicol and Beckett (1985): Cd: 0.349 mmol · kg^{-1} DW, Cu: 0.167 mmol · kg^{-1} DW, Zn: 3.638 mmol · kg^{-1} DW. It may be deduced from the regression equations given in Table 5 that at equilibrium, the NOEC for Cu will be exceeded in soils N (predicted k_x: 0.229 mmol · kg^{-1} DW), O (0.198 mmol · kg^{-1} DW), and P (0.184 mmol · kg^{-1} DW). The NOEC for Zn is expected to be exceeded in soils E (6.916 mmol · kg^{-1} DW), J (4.495 mmol · kg^{-1} DW), X (8.912 mmol · kg^{-1} DW), AB (7.093 mmol · kg^{-1} DW), AK (4.503 mmol · kg^{-1} DW), and AQ (9.698 mmol · kg^{-1} DW). The critical NOEC for Cd will not be exceeded in any of the soils tested. The latter finding supports earlier observations of Chaney (1989) showing that of all metals included in this study, Cd is the only metal that might pose human or animal health risks at plant tissue concentrations that are not generally phytotoxic. Of all metals included in this study, Cd is the metal of most concern due to bioaccumulation through the soil-plant-animal food chain.

On the other hand, from the data given in Tables 1 and 5 it may be deduced that internal Cd and Zn concentrations in soils M, AC, and AE (soils in which abnormalities with regard to plant performance were observed) are predicted to be below the critical NOEC values mentioned. In addition: as the k_x values calculated for these soils are well within the range of experimental Cd and Zn concentrations, the abnormalities observed in the three soils mentioned probably are not due to Cd or Zn toxicity, especially when considering that these soils are quite acidic (pH between 3.49 and 4.63).

Conclusion

The plant root can be regarded as a selective sink for ions in the soil solution. Metals may enter the plant root through a number of pathways. The most important pathway generally is uptake from the soil solution, preceded by transport to the root by either mass flow of water to replace transpirational losses or by diffusion of metal through the solution in response to a concentration gradient induced by selective uptake of metal ion by the root (Barber 1995). The flux of metal to the root is therefore a product of the volume of water absorbed and the concentration of metal in the soil solution. Where metal uptake by the plant roots exceeds the supply brought to the root by mass flow, a depletion of metal in the rhizosphere occurs and metals may diffuse toward the root in response to this concentration gradient. Model calculations of McLaughlin *et al*. (1997) for Cd at low levels of contamination in soil suggested that some depletion of Cd may occur at the root surface. However, extractions carried out at the end of the exposure period employed in the study reported here do not support these calculations. Since, as stated in the introduction section, the soil solution is effectively "emptied" and replenished many times (Bouldin 1989), plant metal uptake is dependent not only on the availability of the metal in the soil solution (*intensity*) and the particularities of the plant's uptake mechanisms but also on the soil's capacity to supply that particular element (*capacity*). Understanding metal bioavailability in soils requires the consideration of the intensity of the toxic exposure and the soil's capacity to maintain this level, in this case in the rhizosphere. In this study it was found that the 0.01 M $CaCl_2$-extractable metal pool provides the best empirical descriptor for the capacity of the soil to supply Cd and Zn. In addition it was found that when expressing the intensity of exposure in terms of exceeding critical plant tissue threshold levels, phytotoxic effects related to excess Zn cannot be ruled out in a number of soils. No abnormalities with regard to plant performance were, however, observed in these soils. The critical NOEC for Cd was not exceeded in any of the soils tested. It may nevertheless be concluded that of all metals included in this study, Cd is the metal of most concern due to bioaccumulation through the soil-plant-animal food chain as Cd is the only metal that might pose human or animal health risks at plant tissue concentrations that are not generally phytotoxic.

$CaCl_2$ extraction is a common extraction technique that is well suited for predicting steady-state tissue metal concentrations in lettuce. Further studies are required as to investigate the universal nature of this extraction technique for predicting metal uptake by a variety of plant species. When coupled to the appropriate critical tissue levels, this would allow for a simple

methodology for assessing potential and actual risks posed by the presence of metals in soil ecosystems that would be well suited in daily practice of risk assessment. Although it cannot be ruled out that other extraction techniques might even provide a better estimate, it is shown here that the methodology in principle is suited for both contaminated field soils and artificially contaminated soils.

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