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Modelling metal accumulation using humic acid as a surrogate for plant roots

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HIGHLIGHTS

- Model total metal concentrations in roots based on metal binding to humic acid.
- Model internalized metal concentrations from metal binding to humic acid.
- Evaluate predictive potential of the WHAM-HA model for modelling root accumulation.
- Review relationship between metal binding to humic acid and root accumulation.

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ABSTRACT

Metal accumulation in roots was modelled with WHAM VII using humic acid (HA) as a surrogate for root surface. Metal accumulation was simulated as a function of computed metal binding to HA, with a correction term (E_{HA}) to account for the differences in binding site density between HA and root surface. The approach was able to model metal accumulation in roots to within one order of magnitude for 95% of the data points. Total concentrations of Mn in roots of *Vigna unguiculata*, total concentrations of Ni, Zn, Cu and Cd in roots of *Pisum sativum*, as well as internalized concentrations of Cd, Ni, Pb and Zn in roots of *Lolium perenne*, were significantly correlated to the computed metal binding to HA. The method was less successful at modelling metal accumulation at low concentrations and in soil experiments. Measured concentrations of Cu internalized in *L. perenne* roots were not related to Cu binding to HA modelled and deviated from the predictions by over one order of magnitude. The results indicate that metal uptake by roots may under certain conditions be influenced by conditional physiological processes that cannot simulated by geochemical equilibrium. Processes occurring in chronic exposure of plants grown in soil to metals at low concentrations complicate the relationship between computed metal binding to HA and measured metal accumulation in roots.

1. Introduction

Consumption of vegetables is one of the most important sources for metal accumulation in humans (Cohen et al., 1998; Swartjes et al., 2007). Considerable amounts of metals can be accumulated in vegetables, subsequently entering the human food chain thus posing potential risks to human health (Redjala et al., 2009). According to Lijzen et al. (2001), exposure via vegetable consumption should be considered in human risk assessment for all metals. So far, vegetable consumption has been included in various exposure models in different countries, e.g., CLEA, RBCA Tool Kit, and CSOIL (DEFRA and EA, 2002; Carlon and Swartjes, 2007; Brand et al., 2007; Conor et al., 2007). In such models, metal concentrations in edible parts of vegetables are usually estimated from empirical bioconcentration factors or regression equations, while soil properties are hardly taken into account or only via rather simplified approaches (Swartjes et al., 2007). These empirical

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methods provide no understanding of underlying processes determining metal accumulation in edible parts of vegetables. In most models, root uptake is not assessed explicitly, although it has been demonstrated to be the most important route for the accumulation of many metals (Smolders, 2001; Malecka et al., 2008). Investigation of metal uptake by plant roots is the first step toward a better understanding of the relationship between human exposure to metals via vegetable consumption and contamination in soil and a mechanistically based modelling approach.

The plant root surface is a heterogeneous mixture of functional groups that are potential binding sites for metals and protons (Fein et al., 1997; Cox et al., 1999; Tiemann et al., 1999; Parsons et al., 2002; Gardea-Torresdey et al., 2002; Ginn et al., 2008). According to Ke and Rayson (1992), it is impossible to adequately simulate ion sorption to the plant root surface without considering specific binding. In addition, the negative charge at the root surface creates an electrical potential (Wagatsuma and Akiba, 1989; Kinraide, 1998). Both the electrostatic interactions and chemical heterogeneity of biological surfaces influence metal root uptake (Kinraide, 2001; Lindberg et al., 2004). These two characteristics of plant root surfaces have also been observed in humic acids (Milne et al., 1995). Humic acids possess a heterogeneous mixture of metal-binding groups, largely carboxylic and phenolic acids (Antunes et al., 2012) with smaller amounts of N- and S-based groups, which display strong affinities for metals. Furthermore, the ratio of 1:2 between the site densities of phenolic and carboxylic groups assumed in WHAM (Tipping, 1998) is within the range reported for root cell walls of different species such as lupine, wheat, and pea, i.e., from 0.49:1 to 1:1 (Meychik and Yermakov, 2001). Because of these similarities, humic acids in humic ion-binding models have been considered a surrogate for biological surfaces such as root cell walls in estimating metal bioaccumulation (Tipping et al., 2008; Antunes et al., 2012; Iwasaki et al., 2013; Tipping and Lofts, 2013). The relevance of using metal binding to HA to represent metal bioaccumulation at biological surfaces is attributable to the nature of metal cation sorption onto biological surfaces (Postma et al., 2005; Antunes et al., 2007). Accordingly, competition among cations, which is considered when computing metal binding to HA, is taken into account in predicting metal accumulation in plant roots. In addition, the available set of binding constants in WHAM facilitates wide application for estimating root uptake of metals in different species. Because of the promising results from using this approach to estimate metal accumulation in bryophytes and metal toxicity to duckweed and macroinvertebrates (Tipping et al., 2008; Antunes et al., 2012; Iwasaki et al., 2013), we aimed to evaluate its potential for modelling metal accumulation in roots of vascular plants. Such root accumulation models might ultimately lead to improved models for predicting human exposure in risk assessment.

In previous studies, binding sites of humic acids have been used to represent the binding sites on reactive surfaces of various organisms (Tipping et al., 2008; Antunes et al., 2012; Iwasaki et al., 2013; Tipping and Lofts, 2013). However, the concentration of metals in roots available for transport to other parts of vegetables depends not only on metal binding to sites at the root surface. After being adsorbed to the root surface, metals might be transported into root cells (internalization) (Campbell et al., 2002; Kalis et al., 2007). Only metals bound to physiologically active sites will be internalized (Campbell et al., 2002). The objective of the present study is to assess the applicability of WHAM with its default binding constants to model both internalized and total (including surface-bound and internalized) metal concentrations in plant roots based on data in the literature. If validated, the use of available binding constants in WHAM allows the WHAM-HA model to be applicable to different species. This initial investigation may provide a basis for further studies on application of the WHAM-HA model for estimating metal accumulation in roots, and hence its applicability in exposure assessment models.

2. Methods

2.1. Data sets

Our simulations of total and internalised metal concentrations in plant roots used data provided by Kopittke et al. (2011), Wu (2007), and Kalis (2006). In the hydroponic studies of Kopittke et al. (2011) and Wu (2007), total metal concentrations in roots exposed to metals in solutions were measured. Effects of major cations, but not organic matter (no humic acid was added to the nutrient solution), were taken into account in determining metal accumulation in plant roots. Kopittke et al. (2011) investigated total concentrations of Mn in roots of *Vigna unguiculata*. The exposure solutions contained varying concentrations of Al (0–10 mM), Ca (0–20 mM), Mg (0–15 mM), and Na (0–20 mM) (Kopittke et al., 2011). Wu (2007) examined total concentrations of Cd, Cu, Ni, and Zn in roots of *Pisum sativum* following exposure to binary mixtures of these metals with Ca in the range from 0 to 2 mM (i.e., Cd–Ca; Cu–Ca; Ni–Ca; and Zn–Ca). Kalis (2006) measured the concentrations of Cd, Cu, Ni, Pb, and Zn that are metabolically taken up (internalized) by roots of *Lolium perenne* exposed to mixtures of these metals in pot experiments. In the experiments, the plants were grown on the soil directly taken from the field, rather than spiking soil with metal salts. The concentration of dissolved organic carbon (0.01 M $CaCl_2$ extraction) in soil varied from 8 to 16 mg L^{-1}. Further information on the data sets, e.g., pH and exposure duration, is given in Table 1.

2.2. Bioaccumulation modelling

In the present study, WHAM VII was used for modelling metal accumulation in plant roots (Tipping, 1994; Tipping et al., 2011). In WHAM, metal sorption to humic substances is simulated by using a structured formulation of discrete, chemically-plausible,

Table 1
Overview of studies used to investigate metal bioaccumulation.

Experiments	Species	Metal	*n*	Exposure concentration (μM)	Exposure duration	pH	Input: Competing cation	Input: Metal species	Output	Studies
Hydroponic cultures	*Vigna unguiculata*	Mn	120	0–1515	48 h	4–6	Ca, Mg, Na, Al	Total concentration	Total root concentration	Kopittke et al. (2011)
Hydroponic cultures	*Pisum sativum*	Cu Ni Zn Cd	45 45 54 45	0–25 0–50 0–140 0–65	48 h	4–6	Ca	Total concentration	Total root concentration	Wu (2007)
Pot (field soil)	*Lolium perenne*	Cd, Ni, Pb, Cu, Zn	50		7 weeks	4–7.1	Al, Fe, Ca, K, Mg, Na	Free metal ion concentration measured in porewater	Internalized concentration	Kalis (2006)

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