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Metal accumulation in the earthworm *Lumbricus rubellus*. Model predictions compared to field data

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Earthworm metal concentrations are less than linearly related to total soil concentrations and predicted pore water concentrations.

Abstract

The mechanistic bioaccumulation model OMEGA (Optimal Modeling for Ecotoxicological Applications) is used to estimate accumulation of zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb) in the earthworm *Lumbricus rubellus*. Our validation to field accumulation data shows that the model accurately predicts internal cadmium concentrations. In addition, our results show that internal metal concentrations in the earthworm are less than linearly (slope < 1) related to the total concentration in soil, while risk assessment procedures often assume the biota-soil accumulation factor (BSAF) to be constant. Although predicted internal concentrations of all metals are generally within a factor 5 compared to field data, incorporation of regulation in the model is necessary to improve predictability of the essential metals such as zinc and copper.

Keywords: Metal; Bioaccumulation model; *Lumbricus rubellus*; Field data

1. Introduction

Ecological risk assessment of metal-polluted ecosystems is commonly based on total soil metal concentrations (Crommentuijn et al., 2000; Lock and Janssen, 2001). However, total concentrations do not necessarily indicate bioaccumulation and toxicity of metals to biota. In the first place, soil specific parameters as pH and organic matter content can strongly determine the chemical availability of metals in soils (Sauvé et al., 2000). Secondly, the metal fraction available for uptake by biota is dependent on the exposure route. Soft-bodied, soil-dwelling organisms are exposed to metals either through direct dermal contact with metals in soil solution or by ingestion of bulk soil or specific soil fractions (Lanno et al., 2004). Finally, some species may limit bioaccumulation of some metals by active excretion and/or reducing uptake,

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thereby maintaining low metal body burdens even at high exposure concentrations (Rainbow, 2002). Most species, however, cannot regulate bioaccumulation of non-essential metals (Rainbow, 2002; Cain et al., 2004). These organisms may prevent toxicity by effectively storing metals in non-toxic forms, i.e. bound to metal-binding proteins like metallothionein (MT) or incorporated in non-soluble granules (Vijver et al., 2004). Species relying on sequestration as detoxification mechanism may excessively accumulate metals with increasing exposure concentrations without suffering toxic effects. Adverse effects may occur, however, when the capacity of the detoxification mechanism is exceeded (Lock and Janssen, 2001; Rainbow, 2002).

Consequently, information of metal accumulation studies may differ widely, depending on the ecosystems, species and conditions (lab, field) investigated. Bioaccumulation models provide insight in metal- and species-specific differences in accumulation kinetics and facilitate interpretation of field data. Additionally, models allow prediction for other cases. Various bioaccumulation models have been developed for aquatic (Luoma and Rainbow, 2005; Steen Redeker et al., 2004) and terrestrial species (Saxe et al., 2001; Heikens et al., 2001). Here, we use the OMEGA (Optimal Modeling for Ecotoxicological Applications) model developed for studying accumulation of metals and neutral organic substances in terrestrial and aquatic food chains. OMEGA has been successfully applied to estimate accumulation of hazardous substances in freshwater, marine and terrestrial communities (Hendriks et al., 2001; Hendriks and Heikens, 2001; Veltman et al., 2005, 2006). The added value of OMEGA in comparison to most other bioaccumulation models is that estimations of uptake and elimination rate constants are based on allometric and biochemical transport principles. This facilitates extrapolation to other soils, contaminant levels, and species and even other metals, without data intensive and case specific calibration.

The aim of the present investigation is two-fold. On the one hand, we want to validate OMEGA for accumulation of zinc, copper, cadmium and lead in the earthworm *Lumbricus rubellus*, thriving in floodplains. On the other hand, we want to check whether field data from a large monitoring program are consistent with data sets on which the model was calibrated. Elimination kinetics were modeled following two approaches. Firstly, a maximum elimination rate was obtained by adding the rate constants of excretion, egestion and "biomass dilution". Secondly, earthworms may sequester metals in an irreversible form, yielding a minimum elimination rate, assuming release via biomass dilution only. Pore water-mediated dermal uptake of metals is assumed to be the dominant exposure route for earthworms, based on research of Vijver et al. (2003) and Saxe et al. (2001). Vijver et al. (2003) showed that earthworms with sealed mouths accumulated almost equal amounts of metals as "normal" earthworms, suggesting that uptake via the skin is the main exposure route. We did not model dietary uptake of metals due to lack of information on the metal fraction in the organic matter phase of solids, for most metals, and the (metal-specific) assimilation efficiency from the gut of this fraction. In our mechanistic model approach, this information is required, as it is known that earthworms selectively feed on specific soil fractions that are rich in organic matter (Bolton and Phillipson, 1976). Internal concentrations predicted by OMEGA were compared to field accumulation data in earthworms from different floodplain soils in the Netherlands. To justify future application of the model for different soil types or other areas, monitoring data from various locations in the Netherlands were included too ($n = 9-15$ locations, see Supporting data). We studied the essential metals, copper and zinc, and the non-essential metals, cadmium and lead. Regression analysis was used to determine the relationship between earthworm metal concentrations and external concentrations. The latter comprises both total soil levels and pore water concentrations.

2. Methods

2.1. OMEGA

OMEGA estimates accumulation of metals in biota as a function of the exposure concentration of the substance and the weight and trophic level of the species. Here, a brief explanation of main processes and basic equations of OMEGA is given. More detailed information can be found in Hendriks et al. (2001) and Hendriks and Heikens (2001).

Earthworms predominantly accumulate metals via pore water-mediated dermal uptake (Vijver et al., 2003; Saxe et al., 2001). Ingestion of soil is excluded as a route of metal uptake for earthworms and steady-state internal concentrations are calculated as the influx via water (absorption), divided by total elimination rate (sum of excretion with water, egestion with faeces and growth dilution) (Eq. (1)).

$$C_{i,x} = \frac{k_{x,\mathrm{w,in}} C_{0\mathrm{w},x}}{k_{x,\mathrm{w,ex}} + k_{x,n,\mathrm{ex}} + k_{x,r}} \tag{1}$$

where $C_{i,x}$ = metal concentration in *L. rubellus* [kg kg^{-1} wet weight], $C_{0\mathrm{w},x}$ = metal concentration in pore water [kg L^{-1}], $k_{x,\mathrm{w,in}}$ = metal absorption rate constant [kg kg^{-1} wet wt d^{-1}/kg L^{-1}], $k_{x,\mathrm{w,ex}}$ = metal excretion rate constant [d^{-1}], $k_{x,n,\mathrm{ex}}$ = metal egestion rate constant [d^{-1}], $k_{x,r}$ = dilution rate constant [kg kg^{-1} wet wt d^{-1}/kg L^{-1}], 'x' represents different metals.

Rate constants for influx and efflux are correlated to species weight by allometric regressions. Additionally, these constants are inversely proportional to resistances that substances encounter in water and lipid layers and flow delays (Hendriks et al., 2001; Hendriks and Heikens, 2001). Adjacent coefficients and parameters have been calibrated on hundreds of rate constants from laboratory studies and typical values are used (Hendriks et al., 2001; Hendriks and Heikens, 2001).

2.2. Uptake and elimination

In contrast to neutral organic substances, predicted uptake rate constants of metals depend on the exposure concentration (for example: Lock and Janssen, 2001). Metals are transported through membranes by protein-carriers or protein-channels (Bryan, 1984; Foulkes, 2000; Hendriks and Heikens, 2001). At high external concentrations, availability of these carriers may become limited and uptake rates will decrease. Therefore, lipid layer resistance for influx via absorption is defined as a function of the exposure concentration (Eq. (2)), analogous to Michaelis–Menten kinetics for enzymes or Langmuir kinetics for sorption.

By movement through moist soils earthworms efficiently exchange substances with water via their skin. Hence, the total delay imposed by the water flux in earthworms is less than that in hard-bodied organisms where exchange with water is limited by drinking and excretion. This is accounted for by using

a value of 200 for γ_0 (similar to aquatic species), instead of the typical value of 0.2 for terrestrial species (Hendriks and Heikens, 2001).

$$k_{x,\mathrm{w,in}} = \frac{1}{\left(\rho_{\mathrm{H_2O,0}} + \rho_{\mathrm{CH_2,w,in}} C_{0\mathrm{w},x}^{\kappa_p} + \frac{1}{\gamma_0}\right) w^{\kappa}} \tag{2}$$

where $\rho_{\mathrm{H_2O,0}}$ is the water layer diffusion resistance (2.8×10^{-3}) [d kg$^{-\kappa}$]; $\rho_{\mathrm{CH_2,w,in}}$ is the lipid layer resistance for influx of metals from water (1.0×10^{-3}) [d kg$^{-\kappa}$]; γ_0 is the water absorption–excretion coefficient (200) [kg$^{\kappa}$ d^{-1}]; κ is the rate exponent (0.25) [−]; κ_p is the lipid layer resistance exponent (0.41) [−]; w is the species wet weight (2.6×10^{-3}) [kg].

Elimination of metals may occur via three different pathways: excretion with water (Eq. (3)), egestion with faeces (Eq. (4)) and dilution with biomass (Eq. (5)). Egestion with faeces is included in the model, as metals may be egested with faeces, irrespective of dietary uptake. Biomass dilution includes individual growth as well as reproduction and replacement of tissues. Generally, metals are excreted slowly if they are bound to metal-binding proteins in soft tissues and/or incorporated in hard tissues, such as shells, feathers, and fur (Hendriks and Heikens, 2001). Binding to dry tissues is incorporated in OMEGA as a generic tissue water distribution coefficient (K_{tw}). This coefficient describes the affinity of metals for dry tissue and is derived from calibration on thousands of metal accumulation ratios from laboratory and field studies (Hendriks and Heikens, 2001).

$$k_{x,\mathrm{w,ex}} = \frac{1}{K_{\mathrm{tw}} p_{s,i}} \frac{1}{\left(\rho_{\mathrm{H_2O,0}} + \rho_{\mathrm{CH_2,ex}} + \frac{1}{\gamma_0}\right) w^{\kappa}} \tag{3}$$

$$k_{x,n,\mathrm{ex}} = \frac{1}{K_{\mathrm{tw}} p_{s,i}} \frac{1}{\left(\rho_{\mathrm{H_2O,faeces}} + \rho_{\mathrm{CH_2,ex}} + \frac{1}{K_{\mathrm{tw}} p_{s,i-1} (1 - f_{\mathrm{as}}) \frac{(\gamma_2 + \gamma_{\mathrm{resp}} q_{\mathrm{ap}})}{f_{\mathrm{as}}}}\right) w^{\kappa}} \tag{4}$$

$$k_{x,r} = \gamma_2 w^{-\kappa} \tag{5}$$

where k_{food} is the food ingestion rate constant [kg kg^{-1} d^{-1}]; K_{tw} is the dry-tissue water partition coefficient (8.0×10^3) [kg kg^{-1} dry wt/kg L^{-1}]; f_{as} is the fraction of food assimilated (40%) [−]; $p_{s,i}$ is the dry weight fraction of *L. rubellus* (i) (15%) [kg dry wt/kg wet wt]; $p_{s,i-1}$ is the dry weight fraction of diet (i−1) (10%) [kg dry wt/kg wet wt]; $\rho_{\mathrm{CH_2,ex}}$ is the lipid layer resistance for efflux of metals (0.3) [d kg$^{-\kappa}$]; $\rho_{\mathrm{H_2O,faeces}}$ is the water layer resistance to faeces (1.1×10^{-5}) [d kg$^{-\kappa}$]; γ_2 is the biomass (re)production coefficient (0.00075) [kg$^{\kappa}$ d^{-1}]; γ_{resp} is the average respiration rate coefficient (0.00075) [kg$^{\kappa}$ d^{-1}]; q_{ap} is the animal to plant respiration coefficient (6.0) [−].

Uptake rate constants are assumed to be metal independent because empirical absorption rates show little metal-specific variation when averaged over different species (Hendriks and Heikens, 2001). Earthworms rely on sequestration to prevent damage of metals (Vijver et al., 2006). This affects metal elimination kinetics because strongly bound substances are likely hardly eliminated via excretion or egestion. Therefore, elimination is modeled in two ways: firstly a maximum elimination rate is calculated as the sum of excretion, egestion and dilution with biomass. Secondly, a minimum elimination rate is obtained by assuming that metals are only eliminated by ''dilution with biomass''. In other words, tight binding to proteins or storage in detoxified forms is incorporated in the model, by increasing the value of K_{tw} (Eqs. (3) and (4)). As a result, excretion and egestion rate constants (Eqs. (3) and (4)) approximate zero, and the total modeled elimination rate equals the rate for biomass dilution. These maximum and minimum elimination rates are assumed to be uniform constants for all metals. The approach results in two estimated internal concentrations for each soil concentration.

OMEGA requires dissolved pore water concentrations to predict internal metal concentrations. Pore water concentrations are estimated using a semi-mechanistic adsorption model that accounts for pH, total soil metal concentrations and soil organic matter content (Sauvé et al., 2000). This adsorption model was derived from a large variety of soils, with a pH range that includes the values noted in our soils (Sauvé et al., 2000). The pore water concentrations predicted by the Sauvé model are comparable to estimated levels using solid-solution partitioning regressions specific for floodplain soils (data not shown) (Schröder et al., 2005) (K_p). The model of Sauvé et al. (2000) was used instead of the Schröder regressions, as we included accumulation data of non-floodplain soils (see Supporting data) and this generic model allows extrapolation to other soil types.

2.3. Data collection and treatment

Field data on the accumulation of metals in the earthworm species *L. rubellus* and total soil concentrations were collected from five studies: Hendriks et al. (1995), Hobbelen et al. (2004), Van Vliet et al. (2005), Koolhaas (unpublished data) and Peijnenburg (unpublished data). The first four studies comprise accumulation data of different Dutch floodplain soils, namely two locations of the river Rhine delta (Gelderse Poort and Ochten) (Hendriks et al., 1995), the Biesbosch (Hobbelen et al., 2004; Koolhaas (unpublished data)), and the Afferdensche and Deestsche Waarden (Van Vliet et al., 2005). The data of Peijnenburg represent different locations in the Netherlands, including various soil types.

95%-Confidence intervals were obtained using samples from the same location. For Biesbosch data these confidence intervals represent the ''individual'' variability in metal concentrations in *L. rubellus*. For data of Peijnenburg the 95%-confidence intervals represent the seasonal variability in earthworm metal body concentrations. Information on variation in total soil concentrations, pH and organic matter fraction is provided in the Supporting data.

Analytical procedures were carried out as described in previous papers (Hendriks et al., 1995; Hobbelen et al., 2004; Van Vliet et al., 2005). Analytical methods used by Koolhaas are described in Hobbelen et al. (2004). For data of Peijnenburg, soils were characterised in terms of pH(pw) and loss-on-ignition (indicated as: LOI, in units of: %). LOI is considered representative of the organic matter content of the solid phase and was determined from the weight loss of approximately 5 g of dried soil (105 °C), heated at 550 °C for 3 h. pH(pw) was determined directly in the pore water, with a pH glass electrode Sentix® stored in a buffer solution, employing an electronic voltmeter (pH meter) against a saturated solution of KCl.

For metal analysis in soils by Peijnenburg, about 1.0 g of ground air-dry soil was weighed into a microwave digestion bomb and 4 ml concentrated nitric acid and 12 ml concentrated hydrochloric acid were added to each bomb. The soil samples were digested in a microwave oven (CEM Corporation – MDS 2000) for 1 h at 180 psi. Following cooling of the samples, the solution was quantitatively transferred into a volumetric flask and diluted to a final volume of 100 mL with milli-Q water. This solution was passed through a 0.45-μm filter. For reference purposes, seven blanks and seven standard soils were digested simultaneously. Metal cation concentrations in the sieved solution were determined by ICP-AES (Spectro Analytical Instruments, Kleve Germany). Adult, clittelate, earthworms were collected by means of hand sorting from the top 10 cm layer of the soil. They were allowed to void their guts on wetted filter paper during 48 h in the dark at 15 °C. Subsequently, the worms were euthanized at −18 °C and stored at this temperature until they were thawed and dried on a paper tissue. Earthworm tissues were digested overnight in concentrated HNO_3 at 100 °C, after which the remaining acid was removed by boiling. The remaining material was dissolved in 0.1 M HNO_3 and metal concentrations in the digest were determined by means of ICP-AES (Spectro Analytical Instruments, Kleve, Germany). Average dry weight of the organisms (15%) was determined by means of freeze-drying 10 species during 48 h.

In the Afferdensche and Deestsche Waarden and some locations in the Biesbosch, soil metal contents were determined at different depths. As *L. rubellus* is an epigeic, i.e. surface-dwelling earthworm (Bouché, 1977), total metal concentrations in the top-soil layer (0–10 cm) were included from these studies.

The adsorption model requires organic carbon content, soil solution pH and total metal concentration in soils, to estimate the metal pore water concentration. In all studies, total metal concentrations in soil were measured in *aqua regia* extracts. Measured organic matter (OM) fractions were converted to organic carbon fractions assuming 17% of the OM-fraction in soils exists of organic carbon (EC, 2004). pH-$CaCl_2$ was measured in each study except for

Hendriks et al. (1995). Pore water pH was calculated from these pH-$CaCl_2$ according to Eq. (6) (Peijnenburg et al., 2001). For data from Hendriks et al. (1995) a pore water pH-value of 7 was used, which is the average pH for floodplain soils (Schröder, 2005).

$$pH_{pw} = \frac{pH_{CaCl_2}}{1.13} + 1.02 \qquad (6)$$

where pH_{pw} is the pore water pH [−]; pH_{CaCl_2} is the $CaCl_2$ pH [−].

Linear regression analysis was performed to relate earthworm metal concentrations to both total soil levels and estimated pore water concentrations. The regression equations were optimized using a linear least squares fit to find appropriate values for the slope (a) and intercept (b) of the regressions. Apart from the regression parameters a and b, the correlation coefficient (r^2) and the residual standard error (SE) were derived. All data were log-transformed in order to normalize their distribution. To compare empirical regression equations with predictions of OMEGA, model equations were rewritten in a form similar to the regression equations.

3. Results

3.1. Empirical data

Figs. 1 and 2 show that earthworm metal concentrations are not linearly related (slope < 1) to total soil concentrations and estimated pore water concentrations. Compared to the non-essential metal cadmium, internal concentrations of zinc and copper increase slightly with increasing total soil concentrations (Fig. 1a and b). The slope of the regression line is approximately 0.27 for both metals. A relatively steep slope (>0.43) is observed for cadmium and lead (Fig. 1c and d). Zinc concentrations in *L. rubellus* range from 500 to 1906 mg kg^{-1} dry body weight, except of one high concentration of 3653 mg kg^{-1} dry body weight. Substantially lower internal levels are observed for copper, cadmium and lead (Fig. 1b, c and d). Copper concentrations range between 6 and 72 mg kg^{-1} dry body weight. Cadmium concentrations in *L. rubellus* vary between 2 and 154 mg kg^{-1} dry body weight and lead concentrations are within 3 and 132 mg kg^{-1} dry body weight.

A significant correlation is found between earthworm metal concentrations and total soil concentrations ($p < 0.001$) (Table 1). Particularly, for copper and cadmium the explained variance is relatively high (r^2 of 0.59 and 0.65, respectively). In addition, there is a statistically significant relationship between internal concentrations of the metals Zn, Cd, Cu and Pb, and estimated pore water concentrations ($p < 0.05$) (Table 2), with an explained variance between 11 and 47%. The explained variance found for lead is relatively low ($r^2 = 0.11$) compared to the other metals.

3.2. Model predictions

Accumulation of cadmium in *L. rubellus* is accurately predicted by OMEGA using a minimum elimination rate. The deviations between model estimations and field data are within

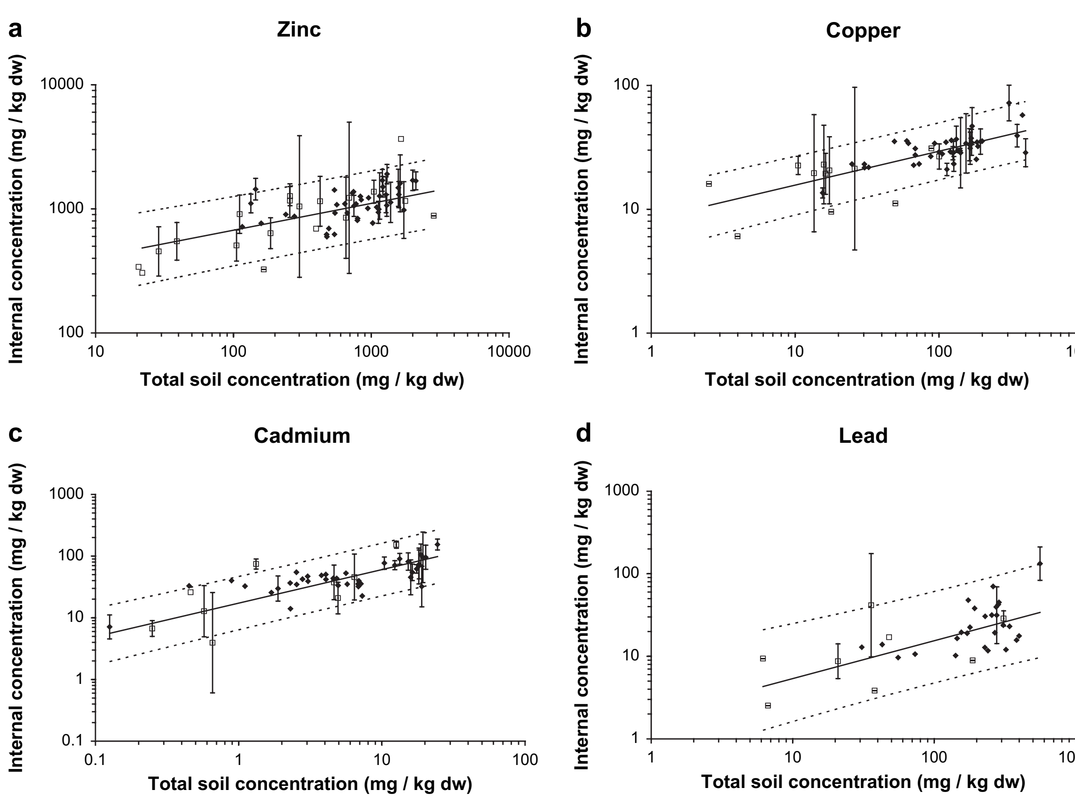

Fig. 1. Measured internal metal concentrations in *Lumbricus rubellus* (C_i, mg kg^{-1} dry body weight) plotted against measured total soil concentrations (C_{soil}, mg kg^{-1} dry soil). ◆ data from Hendriks et al. (1995), Hobbelen et al. (2004), Koolhaas (unpublished data) and Van Vliet et al. (2005) (Rhine, Biesbosch, Afferdensche and Deestsche Waarden,), unpublished data from Peijnenburg (various locations in the Netherlands), full line represents regression. Dashed lines represent 97.5th and 2.5th percentile of the field data. 95% confidence intervals are plotted when possible. For three data points confidence intervals were too large to plot in the figure. These data points (C_{soil}, C_i) are: cadmium (0.46, 25.9), lead (48.1, 17.1), copper (380, 57.6).

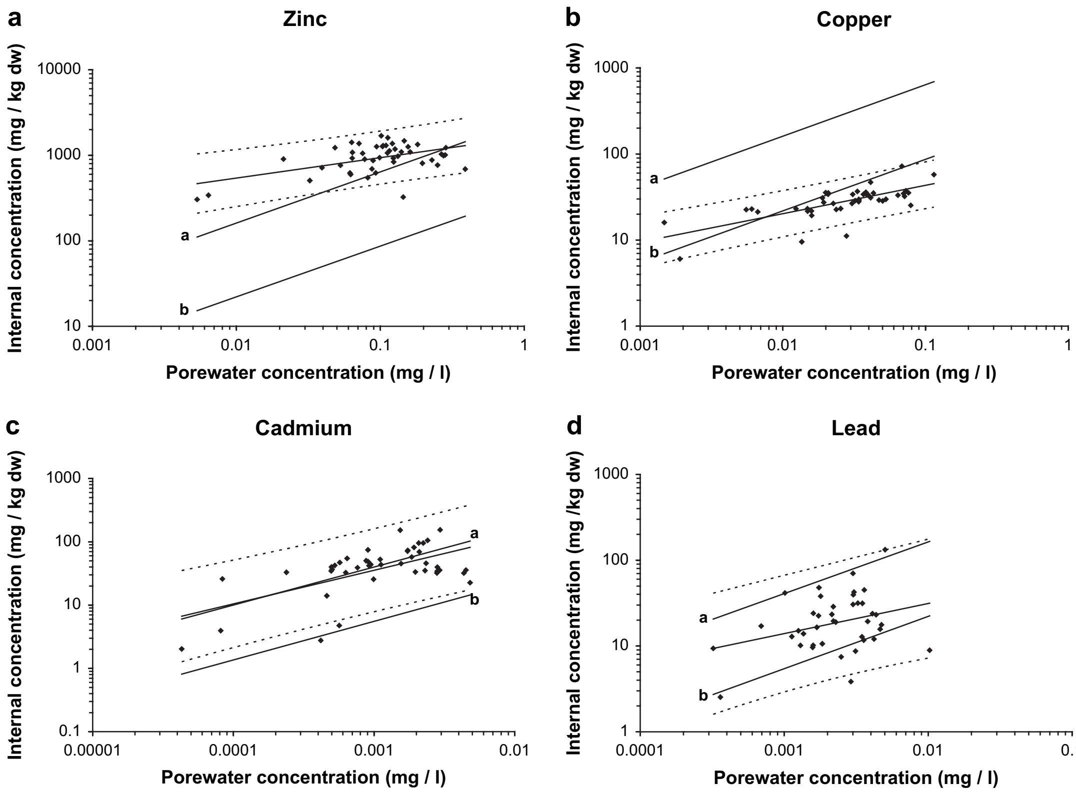

Fig. 2. Measured internal concentrations in *Lumbricus rubellus* (C_i, mg kg^{-1} dry body weight) plotted against estimated pore water concentrations (C_{pw}, mg L^{-1}), compared to OMEGA model predictions. Full line represents regression for field data. The full lines (a) and (b) represent, respectively, (a) minimum elimination (growth dilution only) and (b) maximum elimination (sum of egestion, excretion and growth dilution). Dashed lines represent 97.5th and 2.5th percentile of the field data. See text for further explanation.

a factor 3 (with exception of two data points, which are within a factor 8) (Fig. 2c). The slope of the empirical regression line is comparable to the slope of the model line, i.e. 0.53 versus 0.60, respectively. A large variation in internal lead concentrations is observed (Fig. 2d) and model predictions with both maximum and minimum elimination rate are within the 97.5th and 2.5th percentile of the field data. For both essential metals, the slope of the empirical regression line is lower than expected by OMEGA, i.e. 0.23 for Zn and 0.33 for Cu compared to a value of 0.60 predicted by the model. Earthworm zinc concentrations are underestimated by OMEGA at low pore water concentrations, with a maximum factor of 3 (Fig. 2a). Internal copper concentrations in *L. rubellus* are best predicted using a maximum elimination rate (Fig. 2b).

Table 1
Correlation analysis for internal metal concentrations in *Lumbricus rubellus* (C_i, mg kg^{-1} dry body weight) with total soil concentrations (C_s, mg kg^{-1} dry soil), using data from Hendriks et al. (1995), Hobbelen et al. (2004), Van Vliet et al. (2005), Koolhaas (unpublished data) and Peijnenburg (unpublished data)

Metal	Log (C_i)	r^2	SE	*P*-value
Zn[a]	$0.27\,\mathrm{Log}(C_s) + 2.3$	0.49	0.19	<0.001
Cd	$0.54\mathrm{Log}(C_s) + 12.3$	0.65	0.24	<0.001
Cu	$0.28\,\mathrm{Log}(C_s) + 0.91$	0.59	0.11	<0.001
Pb	$0.43\,\mathrm{Log}(C_s) + 0.38$	0.41	0.25	<0.001

[a] One data point ($C_s = 2100$ mg kg^{-1} dry soil, $C_i = 155$ mg kg^{-1} dry body weight) was identified as an outlier, and therefore excluded from the regression analysis.

4. Discussion

4.1. Empirical regressions

Our results show that internal metal concentrations are statistically significantly correlated to total metal concentrations in soil, as observed in several other studies (Hobbelen et al., 2004; Marinussen et al., 1997; Becquer et al., 2005; Dai et al., 2004). The field data, especially of cadmium and lead, show a relatively large scattering, which partly results from including accumulation data from various soil types, with significant differences in pH and organic matter content (data of Peijnenburg). This results in differences in bioavailability of metals and consequently, variance in accumulation.

The significant relationship between internal concentrations in earthworms and total soil levels, is not necessarily contradicting the assumption that uptake via the skin is the major exposure route. Total soil concentrations are relatively stable in time and may therefore be a good predictor of metal accumulation in chronically exposed earthworms via pore water. Additionally, a statistically significant relationship between internal concentrations and estimated pore water concentrations is observed for all metals. Coefficients of determination (r^2) are somewhat lower for the relation between internal metal concentration in *L. rubellus* and estimated pore water concentrations than for the relationship with total soil levels.

Table 2
Correlation analysis for measured internal metal concentrations in *Lumbricus rubellus* (C_i, mg kg^{-1} dry body weight) with estimated pore water concentrations (C_{pw}, mg L^{-1})

Metal		Log (C_i)	r^2	SE	P-value
Zn[a]	Empirical	$0.23\ \text{Log}(C_{pw}) + 3.19$	0.22	0.15	<0.001
Cd	Empirical	$0.53\ \text{Log}(C_{pw}) + 3.14$	0.37	0.32	<0.001
Cu	Empirical	$0.33\ \text{Log}(C_{pw}) + 2.0$	0.47	0.13	<0.001
Pb	Empirical	$0.37\ \text{Log}(C_{pw}) + 2.24$	0.11	0.31	0.03
[Me]	OMEGA (min. elimination)	$0.60\ \text{Log}(C_{pw}) + 3.4$			
	OMEGA (max. elimination)	$0.60\ \text{Log}(C_{pw}) + 2.6$			

Also included are regression equations estimated with the OMEGA model, using minimum and maximum elimination. See text for further explanation.
[a] One data-point ($C_{pw} = 0.45$ mg L^{-1}, $C_s = 2100$ mg kg^{-1} dry soil, $C_i = 155$ mg kg^{-1} dry body weight) was identified as an outlier, and therefore excluded from the regression analysis.

This probably arises from additional uncertainty introduced by estimating pore water concentrations with the adsorption model. Furthermore, bioavailability of metals is influenced by speciation and various metal species may have different contributions to the total uptake (Chuang and Wang, 2006; Vink, 2002). Regressions may therefore be improved considering different metal species instead of total dissolved pore water concentrations.

4.2. Model predictions

Fig. 2 shows that OMEGA accurately predicts Cd accumulation using a minimum elimination rate and that internal Cu concentrations are in reasonable agreement with model predictions. However, for Zn and Pb the model has less predictability. Deviations between model estimations and field data may result from the assumption that total dissolved metal concentrations are bioavailable for uptake by earthworms, whereas Chuang and Wang (2006) showed that various metal species have different contributions to the total uptake. Also predicting pore water concentrations by the Sauvé model may explain differences between model estimations and field data. We compared pore water concentrations estimated with the Sauvé model with those predicted by K_p-regressions developed for floodplain soils (Schröder et al., 2005) (data not shown). The predicted pore water concentrations are comparable for cadmium and copper, i.e. within a factor 2 (excluding one exception for Cd which is within a factor 5). Model predictions, and conclusions, are not influenced using Schröder regressions instead of the Sauvé model, for cadmium, copper and zinc. However, for lead, pore water concentrations estimated with Schröder regressions tend to be lower (factor 5–12) than predictions with the Sauvé model. A low coefficient of determination ($r^2 = 0.10$) is found for the relation between earthworm lead levels and estimated pore water concentrations. This may partly be attributed to the lower predictability of lead pore water concentrations compared to other metals (Sauvé et al., 2000). Due to the large variability in internal concentrations and the lower predictability of pore water concentrations conclusions on lead accumulation cannot be drawn.

For the essential metals zinc and copper, the slope of the field regression line is lower than expected by OMEGA, i.e. approximately 0.30 versus 0.60. This can be explained by regulation of internal concentrations of these metals. Regulation of zinc has been observed in different earthworm species, *Eisenia fetida* (Spurgeon and Hopkin, 1999; Lock and Janssen, 2001), *Eisenia andrei* (Peijnenburg et al., 1999; Van Gestel et al., 1993) and *L. rubellus* (Ireland, 1979). Copper is one of the more toxic essential metals (Finney and O'Halloran, 2003). Therefore, it is of utmost importance for species to effectively detoxify and/or regulate internal copper levels above metabolic requirements. Morgan and Morgan (1990) concluded that *L. rubellus* is not able to sequester Cu by metal-binding ligands in some tissues. To compensate this lack of a storage mechanism the earthworm probably invests in regulating uptake or elimination rates of copper, which may explain the slight increase in earthworm copper concentrations with increasing total soil concentrations and increasing pore water levels.

Even at low exposure concentrations *L. rubellus* contains relatively high zinc levels compared to levels of other metals. At these low external concentrations, internal zinc concentrations are underestimated by OMEGA. This may result from either underestimation of the uptake rate or overestimation of the elimination rate. The latter explanation is unlikely as the minimum modeled elimination rate is already rather low, i.e. 3.3×10^{-3} d^{-1}. Vijver et al. (2003) and Saxe et al. (2001) showed that 20–30% of internal zinc concentrations resulted from ingestion of soil, whereas for the other metals, Cd, Cu and Pb, uptake could be completely attributed to absorption via the skin. Therefore, excluding ingestion of soil may not be valid for zinc, and total uptake rate constants, i.e. absorption plus ingestion, may be underestimated.

4.3. Elimination rates

Internal concentrations of cadmium are best predicted assuming that this metal is only eliminated by growth dilution, although a relatively high variation in internal Cd concentrations is observed. From immunohistochemical observations it is known that a significant proportion (>70%) of the earthworm Cd concentration is sequestered by cysteine-rich metal-binding proteins, such as metallothionein (MT) (Vijver et al., 2006; Morgan et al., 2004; Stürzenbaum et al., 2004,

2001). This detoxification mechanism is highly efficient (Stürzenbaum et al., 2004). Cadmium is tightly bound by MT-2 (isoform 2 metallothionein) and very low elimination rates have been reported ($4.6 \times 10^{-3}\ d^{-1}$ for *Lumbricus terrestris*) (Spurgeon and Hopkin, 1999; Sheppard et al., 1997). These empirical rate constants of loss are in good agreement with the predicted minimum elimination rate of $3.3 \times 10^{-3}\ d^{-1}$.

Comparing elimination rate constants estimated using OMEGA with values reported in literature (Table 3), contradicting results are observed as empirical clearance rates vary as much as two orders of magnitude. For example elimination rate constants reported for copper range between 0.02 and $1.5\ d^{-1}$ (Spurgeon and Hopkin, 1999). Empirical elimination rates are consistent for cadmium only, with low elimination rates reported for most species. Modeled minimum elimination rates are comparable to the lowest values in the range.

For zinc, copper and lead, empirical elimination rate constants may only be used in a relative manner. Apparently, copper and lead are more easily eliminated than cadmium, which is in agreement with our observation that internal concentrations of these metals are best modeled using a maximum elimination rate.

4.4. Relevance for environmental risk assessment

Bioaccumulation is often used as a criterion for prioritization and risk assessment of both organic substances and metals (McGreer et al., 2003). Our validation to field data shows that OMEGA is capable of accurately predicting bioaccumulation of the non-essential metal cadmium in the earthworm *L. rubellus*. In addition, our results show that internal metal concentrations in the earthworm are less than linearly (slope < 1) related to the total concentration in soil, while risk assessment procedures often assume the biota-soil accumulation factor (BSAF) to be constant (Crommentuijn et al., 1997; Lock and Janssen, 2001; McGreer et al., 2003). Obviously, the regressions collected in the present and previous studies can be used to obtain a concentration-dependent accumulation factor. However, the advantage of using a model like the one proposed here is that it facilitates extrapolation to soils of various origins, with different physico-chemical properties, and to other species, with similar physiology and metal detoxification mechanisms as earthworms. An additional application for risk assessment purposes is that model predictions underpin the correctness of field measurements, as field accumulation data can show large variability as well. OMEGA may also be used for other non-essential metals in addition to cadmium. However, the model needs incorporation of regulation to improve predictability for essential metals as zinc and copper. Additionally, the model may be improved by considering metal speciation in pore water, to account for differences in bioavailability between various metal species.

5. Conclusion

Internal metal concentrations in *L. rubellus* are significantly related to total soil concentrations and estimated pore water concentrations. These internal concentrations show a less than linear relationship (slope < 1) with external concentrations. The model accurately predicts accumulation of the non-essential metal cadmium. However, insight in accumulation kinetics of metals, especially regulation of uptake and elimination of essential metals, is necessary to improve the predictability of copper and zinc.

Table 3
Empirical constants for the elimination of zinc, copper, cadmium and lead from different earthworm species reported in the literature

Metal	Species	Elimination rate (d^{-1})	References
Cadmium	*Eisenia andrei*	0.078	Honeycutt et al. (1995)
	Allolobophora tuberculata	0.018	Neuhauser et al. (1995)
	Eisenia andrei	0.032	Peijnenburg et al. (1999)
	Eisenia fetida	0–0.081	Spurgeon and Hopkin (1999)
	Lumbricus terrestris	0.0046	Sheppard et al. (1997)
	Geometric mean[a]	0.03	
Zinc	*Allolobophora tuberculata*	0.034	Neuhauser et al. (1995)
	Eisenia fetida	0–1.84	Spurgeon and Hopkin (1999)
	Lumbricus terrestris	0.01	Sheppard et al. (1997)
	Geometric mean[a]	0.25	
Copper	*Eisenia fetida*	0–1.63	Spurgeon and Hopkin (1999)
	Lumbricus rubellus	0.04–0.95	Marinussen et al. (1997)
	Geometric mean	0.37	
Lead	*Eisenia andrei*	1.2	Peijnenburg et al. (1999)
	Eisenia fetida	0.024–0.47	Spurgeon and Hopkin (1999)
	Geometric mean	0.26	
OMEGA	Maximum elimination rate constant	0.02	
	Minimum elimination rate constant	0.003	

Also included are minimum and maximum elimination rates of metals calculated for *Lumbricus rubellus* using the OMEGA model. See text for further explanation.
[a] Elimination rates with a value of '0' are not included in the geometric mean.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2006.06.033.

References

Becquer, T., Dai, J., Quantin, C., Lavelle, P., 2005. Sources of bioavailable trace metals for earthworms from a Zn-, Pb-, and Cd-contaminated soil. Soil Biology and Biochemistry 37, 1564–1568.

Bolton, P.J., Phillipson, J., 1976. Burrowing, feeding, egestion and energy budgets of *Allolobophora rosea* (Savigny) (Lumbricidae). Oecologia 23, 225–245.

Bouché, M.B., 1977. Stratégies lombriciennes. In: Lohm, U., Persson, T. (Eds.), Soil Organisms as Components of Ecosystems. Ecological Bulletin NFR, Stockholm, pp. 122–132.

Bryan, G.W., 1984. Pollution due to heavy metal and their compounds. In: Kinne, O. (Ed.), Marine Ecology, vol. 5. John Wiley, New York, NY, USA, pp. 1289–1430.

Cain, D.J., Luoma, S.N., Wallace, W.G., 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. Environmental Toxicology and Chemistry 23, 1463–1473.

Chuang, C.-Y., Wang, W.-X., 2006. Co-transport of metal complexes by the green mussel *Perna viridis*. Environmental Science and Technology 40, 4523–4527.

Crommentuijn, T., Polder, M.D., Van de Plassche, E.J., 1997. Maximum Permissible Concentrations and Negligible Concentrations for Metals: Taking Background Concentrations into Account. RIVM report 601501001. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

Crommentuijn, T., Sijm, D., de Bruin, J., van den Hoop, M., van Leeuwen, K., van de Plassche, E., 2000. Maximum permissible and negligible concentrations for metals and metalloïds in the Netherlands, taking into account background concentrations. Journal of Environmental Management 60, 121–143.

Dai, J., Becquer, T., Rouiller, J.H., Reversat, G., Bernhard-Reversat, F., Nahmani, J., Lavelle, P., 2004. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. Soil Biology and Biochemistry 36, 91–98.

EC, 2004. European Union System for Evaluation of Substances 2.0 (EUSES 2.0). Prepared for the European Chemicals Bureau by the National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands (RIVM Report no. 601900005).

Finney, L.A., O'Halloran, T.V., 2003. Transition metal speciation in the cell: insights from the chemistry of metal ion receptors. Science 300, 931–936.

Foulkes, E.C., 2000. Transport of toxic heavy metals across cell membranes. Proceedings of the Society for Experimental Biology and Medicine 223, 234–240.

Heikens, A., Peijnenburg, W.J.G.M., Hendriks, A.J., 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution 113, 385–393.

Hendriks, A.J., Ma, W.-C., Brouns, J.J., de Ruiter-Dijkman, E.M., Gast, R., 1995. Modelling and monitoring organochlorine and heavy metal accumulation in soils, earthworms, and shrews in Rhine-Delta floodplains. Archives of Environmental Contamination and Toxicology 29, 115–127.

Hendriks, A.J., van der Linde, A., Cornelissen, G., Sijm, D.T.H.M., 2001. The power of size 1. Rate constants and equilibrium ratios for accumulation of organic substance related to octanol–water partition ratio and species weight. Environmental Toxicology and Chemistry 20, 1399–1420.

Hendriks, A.J., Heikens, A., 2001. The power of size 2. Rate constants and equilibrium ratios for accumulation of inorganic substances related to species weight. Environmental Toxicology and Chemistry 20, 1421–1437.

Hobbelen, P.H.F., Koolhaas, J.E., van Gestel, C.A.M., 2004. Risk assessment of heavy metal pollution for detritivores in floodplain soils in the Biesbosch, The Netherlands, taking bioavailability into account. Environmental Pollution 129, 409–419.

Honeycutt, M.E., Roberts, B.L., Roane, D.S., 1995. Cadmium disposition in the earthworms *Eisenia fetida*. Ecotoxicology and Environmental Safety 30, 143–150.

Ireland, M.P., 1979. Metal accumulation by the earthworms *Lumbricus rubellus*. *Dendrobaena veneta* and *Eiseniella tetraedra* living in heavy metal polluted sites. Environmental Pollution 19, 201–206.

Lanno, R., Wells, J., Conder, J., Bradham, K., Basta, N., 2004. The bioavailability of chemicals in soil for earthworms. Ecotoxicology and Environmental Safety 57, 39–47.

Lock, K., Janssen, C.R., 2001. Zinc and cadmium body burdens in terrestrial oligochaetes: use and significance in environmental risk assessment. Environmental Toxicology and Chemistry 20, 2067–2072.

Luoma, S.N., Rainbow, P.S., 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environmental Science and Technology 39, 1921–1931.

Marinussen, M.P.J.C., van der Zee, S.E.A.T.M., de Haan, F.A.M., 1997. Cu accumulation in *Lumbricus rubellus* under laboratory conditions compared with accumulation under field conditions. Ecotoxicology and Environmental Safety 36, 17–26.

McGreer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J., Green, A., 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment. Environmental Toxicology and Chemistry 23, 1017–1037.

Morgan, J.E., Morgan, A.J., 1990. The distribution of cadmium, copper, lead, zinc and calcium in the tissues of the earthworm *Lumbricus rubellus* sampled from one uncontaminated and four polluted soils. Oecologia 84, 559–566.

Morgan, A.J., Stürzenbaum, S.R., Winters, C., Grime, G.W., Aziz, N.A., Kille, P., 2004. Differential metallothionein expression in earthworm (*Lumbricus rubellus*) tissues. Ecotoxicology and Environmental Safety 57, 11–19.

Neuhauser, E.F., Cukic, Z.F., Malecki, M.R., Loehr, R.C., Durkin, P.R., 1995. Bioconcentration and biokinetics of heavy metals in the earthworm. Environmental Pollution 89, 293–301.

Peijnenburg, W.J.G.M., Baerselman, R., de Groot, A.C., Jager, T., Posthuma, L., van Veen, R.P.M., 1999. Relating environmental availability to bioavailability: soil-type-dependent metal accumulation in the oligochaete *Eisenia andrei*. Ecotoxicology and Environmental Safety 44, 294–310.

Peijnenburg, W., de Groot, A., van Veen, R.P.M., 2001. Experimental and theoretical study on equilibrium partitioning of heavy metals. In: Iskandar, I., Kirkham, M.B. (Eds.), Trace Elements in Soil. Bioavailability, Flux and Transfer. CRC press, Boca Raton, FL, USA, pp. 91–126.

Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environmental Pollution 120, 497–507.

Sauvé, S., Hendershot, W., Allen, H.E., 2000. Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden and organic matter. Environmental Science and Technology 34, 1125–1131.

Saxe, J.K., Impellitteri, A., Peijnenburg, W.J.G.M., Allen, H.E., 2001. Novel model describing trace metal concentrations in the earthworm, *Eisenia andrei*. Environmental Science and Technology 35, 4522–4529.

Schröder, T.J., Hiemstra, T., Vink, J.P.M., van der Zee, S.E.A.T.M., 2005. Modeling of the solid-solution partitioning of heavy metals and arsenic in embanked flood plain soils of the rivers Rhine and Meuse. Environmental Science and Technology 39, 7176–7184.

Schröder, T.J., 2005. Solid-Solution Partitioning of Heavy Metals in Floodplain Soils of the Rivers Rhine and Meuse: Field Sampling and Geochemical Modeling. PhD thesis, Wageningen University, Wageningen, The Netherlands.

Sheppard, S.C., Evendsen, W.G., Cornwell, T.C., 1997. Depuration and uptake kinetics of I, Cs, Mn, Zn and Cd by the earthworm (*Lumbricus terrestris*) in radiotracer-spiked litter. Environmental Toxicology and Chemistry 16, 2106–2112.

Spurgeon, D., Hopkin, S.P., 1999. Comparisons of metal accumulation and excretion kinetics in earthworms (*Eisenia fetida*) exposed to contaminated field and laboratory soils. Applied Soil Ecology 11, 227–243.

Steen Redeker, E., Bervoets, L., Blust, R., 2004. Dynamic model for the accumulation of Cadmium and Zinc from water and sediment by the aquatic oligochaete, *Tubifex tubifex*. Environmental Science and Technology 38, 6193–6200.

Stürzenbaum, S.R., Winters, C., Galay, M., Morgan, A.J., Kille, P., 2001. Metal ion trafficking in earthworms. Identification of a cadmium specific metallothionein. Journal of Biological Chemistry 276, 34013–34018.

Stürzenbaum, S.R., Georgie, O., Morgan, A.J., Kille, P., 2004. Cadmium detoxification in earthworms: from genes to cells. Environmental Science and Technology 38, 6283–6289.

Van Gestel, C.A.M., Dirven-van Breemen, E.M., Baerselman, R., 1993. Accumulation and elimination of cadmium, chromium and zinc and effects on growth and reproduction in *Eisenia andrei* (Oligochaeta, Annelida). Science of the Total Environment Suppl., 585–597.

Van Vliet, P.C.J., van der Zee, S.E.A.T.M., Ma, W.-C., 2005. Heavy metal concentrations in soil and earthworms in a floodplain grassland. Environmental Pollution 138, 505–516.

Vijver, M.G., Vink, J.P.M., Miermans, C.J.H., van Gestel, C.A.M., 2003. Oral sealing using glue; a new method to distinguish between intestinal and dermal uptake of metals in earthworms. Soil Biology and Biochemistry 35, 125–132.

Vijver, M.G., van Gestel, A.M., Lanno, R.P., van Straalen, N.M., Peijnenburg, W.J.G.M., 2004. Internal metal-sequestration and its ecotoxicological relevance – a review. Environmental Science and Technology 38, 4705–4712.

Vijver, M.G., van Gestel, C.A.M., van Straalen, N.M., Lanno, R.P., Peijnenburg, W.J.G.M., 2006. Biological significance of metals partitioned to subcellular fractions within earthworms (*Aporrectodea caliginosa*). Environmental Toxicology and Chemistry 25, 807–814.

Vink, J.P.M., 2002. Measurement of heavy metal speciation over redox gradients in natural water–sediment interfaces and implications for uptake by benthic organisms. Environmental Science and Technology 36, 5130–5138.

Veltman, K., Hendriks, J., Huijbregts, M., Leonards, P., van den Heuvel-Greve, M., Vethaak, D., 2005. Accumulation of organochlorines and brominated flame retardants in estuarine and marine food chains. Field measurements and model calculations. Marine Pollution Bulletin 50, 1085–1102.

Veltman, K., Huijbregts, M.A.J., van den Heuvel-Greve, M.J., Vethaak, A.D., Hendriks, A.J., 2006. Organotin accumulation in an estuarine food chain. Comparing field measurements with model estimations. Marine Environmental Research 61, 511–530.