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Estimating Heavy Metal Accumulation in Oligochaete Earthworms: A Meta-analysis of Field Data

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Ecological risk assessment is an important aspect of land use planning and the management of contaminated land. The ecological risk of polluted soil is strongly associated with the extent of bioavailability of the contamination. A typical example is the fact that a high availability of pollutant chemicals to oligochaete earthworms may lead to a toxic exposure of vermivore species of vertebrate wildlife (e.g., Ma & Talmage 2001). The availability of heavy metals for uptake by earthworms depends on the concentration of free metal ions present in the dissolved soil solution phase (Kieviet & Ma 1991). In principle, therefore, it should be possible to estimate metal concentrations in earthworms from free metal ion concentrations by applying equilibrium partitioning theory. However, models that are able to calculate heavy metal speciation in soil require detailed geochemical data input. Analytical methods to measure free metal ion activity directly in the soil solution are technically complex and do not always yield reliable results, especially in case of low metal concentrations when they may have a poor sensitivity (Kheboian & Bauer 1987; Goldberg 1995). Therefore, from a practical point of view, preference may be given to a straightforward measurement of the total acid-extractable concentration of a metal in soil, which can be done in a reliable and routinely manner. An important draw-back of such an approach, however, is that it does not account adequately for local variations in bioavailability which results in a relatively poor predictive capability for estimating metal accumulation in earthworms (Spurgeon & Hopkin 1996; Oste et al. 2001).

The objective of this study was to generate multiple regression models which are based on total acid-extractable concentrations but still capable of accounting for soil-related variations in metal bioavailability. To this end, a literature screening was carried out of suitable bioaccumulation models for Cd, Cu, Pb or Zn in oligochaetes that have been derived from local field data of total acid-extractable concentrations in conjunction with data on covariables that are relevant to metal adsorption-desorption equilibria. Typical relevant site-related covariables in the regression models would include soil pH, clay and organic matter fractions, cationic exchange capacity, and certain macro-element concentrations. In addition to modeling earthworm metal bioaccumulation, the present article also draws attention to the various practical problems that are associated with field sampling of soil and earthworms and which calls for a rigorous standardisation of sampling methodology.

MATERIALS AND METHODS

Data obtained from studies on metal accumulation in earthworms were rigorously selected according to the following criteria:

1. They must refer to field species, thus excluding laboratory exposures of *Eisenia* species, which are adapted to living in compost heaps and not normally found in field soils. In addition, they have physiological properties that clearly deviate from those of field species (e.g., Ma and Bodt 1993).
2. They must refer to concentrations measured in mature adult specimens, thus excluding the immature juvenile and subadult developmental stages.
3. They must refer to specimens with emptied gut contents, thus excluding the contribution of metals present in intestinal soil residues.
4. Concentrations reported for metals in soil should have been determined by extraction with aqua regia or concentrated nitric acid and measured by atomic absorption spectrophotometry rather than from neutron activation analysis.
5. Concentrations in soil and oligochaetes should refer to dry matter and should at least have been obtained from duplicate measurements.
6. Additional data must have been provided on soil parameters that are relevant to soil metal retention capacity and at least include pH and organic matter content.

Species of oligochaetes were grouped according to the pedo-ecological classification described by Bouché (1977). This classification recognises epigeic and endogeic groups that burrow and feed in the organic top layer and the deeper mineral soil layers, respectively, and the anecics which are larger-sized species that feed at the soil surface during night-time but remain in long vertical semi-permanent burrows during the day. Epigeic species include *Dendrobaena sp.* and *Lumbricus sp.*, whereas the endogeics include *Aporrectodea sp.* and *Allolobophora sp.* Two well-known anecic species are *Lumbricus terrestris* and *Aporrectodea longa.* The anecics were used as a reference class in the multiple regression analyses, equations were thus expressed on the basis of the anecic group with additive terms for the epigeic and endogeic groups. Non-weighted data on concentrations of metal and soil organic matter (OM) were used after logarithmical transformation, as they both departed from a normal distribution.

The data of the selected studies were subjected to a meta-analysis (Hedges & Olkin 1985) applying a random coefficient or mixed model for *i*th metal in *j*th study:

$$\log[\mathrm{Me}]_{w,ij} = u_i + e_{ij} + b_i \log[\mathrm{Me}]_{s,ij} + c_i\,\mathrm{pH}_{ij} + d_i \log[\mathrm{OM}] + f_{1,i}\, I_{epi,ij} + g_{2i}\, I_{endo,ij}$$

where I_{epi} and I_{endo} are indicator variables for, respectively, the epigeic and endogeic groups. Me_w and Me_s are metals in worms and soil, respectively.

RESULTS AND DISCUSSION

Scanning the literature on metal accumulation in field worms showed that most of the information provided on parameters of soil metal retention capacity was restricted to

Table 1. Regression models from the scientific literature.

Model	R^2	Ref
$\log[Cd_W] = 1.14 - 0.079\ pH$	12	1
$\log[Cd_W] = 1.28 + 0.324 \log[Cd_S] - 0.230 \log[pH] - 0.560 \log[OM]$	90	2
$\log[Cd_W] = 5.17 + 0.395 \log[Cd_S] - 0.290\ pH + 0.061\ OM - 0.039\ CEC$	71	3,4
$\ln[Cd_W] = 5.538 + 0.664 \ln[Cd_S] - 0.404\ pH$	48	5
$\log[Cd_W] = 1.25 + 0.477 \log[Cd_S]$	86	6
$\log[Cd_W] = 1.32 + 0.614 \log[Cd_S]$	82	6
$\log[Cu_W] = 0.660 - 0.032\ Ca + 0.470\ K$	18	1
$\log[Cu_W] = 1.48 + 0.194 \log[Cu_S] - 0.310 \log[OM]$	82	2
$\log[Cu_W] = 1.89 + 0.564 \log[Cu_S] - 0.026\ CEC$	75	3,4
$[Cu_W] = 14.9 + 0.344 [Cu_S]$	58	5
$\log[Cu_W] = 0.726 + 0.229 \log[Cu_S]$	32	6
$\log[Cu_W] = 0.327 + 0.487 \log[Cu_S]$	11	6
$\log[Pb_W] = 1.20 - 0.035 \log[Pb_S] - 0.17 \log[pH] + 0.10\ OM - 0.19\ Mg$	64	1
$\log[Pb_W] = 2.65 + 0.897 \log[Pb_S] - 3.56 \log[pH]$	93	2
$\log[Pb_W] = 4.98 + 1.69 \log[Pb_S] - 1.08\ pH - 0.127\ CEC$	55	3,4
$\ln[Pb_W] = 4.16 + 1.13 \ln[Pb_S] - 0.176\ OM - 0.746\ pH$	67	5
$\log[Pb_W] = 0.150 + 1.52 \log[Pb_S] - 0.733 \log[Ca]$	82	6
$\log[Pb_W] = 0.732 + 1.25 \log[Pb_S] - 0.219 \log[Ca]$	76	6
$\log[Zn_W] = 1.86 + 0.250 \log[Zn_S] - 0.643 \log[pH]$	74	2
$\log[Zn_W] = 8.89 + 0.402 \log[Zn_S] - 0.508\ pH - 0.343\ CEC$	79	3,4
$\ln[Zn_W] = 6.79 + 0.343 \ln[Zn_S] - 0.270\ pH$	43	5
$\log[Zn_W] = 2.02 + 0.313 \log[Zn_S]$	53	6
$\log[Zn_W] = 2.13 + 0.213 \log[Zn_S]$	58	6

Ref: 1. Beyer et al. (1987); 2. Corp & Morgan (1991); 3. Ma (1982); 4. Ma (1983); 5. Ma et al. (1983); 6. Morgan & Morgan (1988).

pH and organic matter. Table 1 summarises the various regression models that have been derived from these studies. Beyer et al. (1982) studied *Aporrectodea tuberculata* in four agricultural silt loam soils with low-level soil contamination in the United States. Concentrations of Cd were mostly below the detection limit of 0.3 mg/kg. Cu levels were 3.6-11 mg/kg, Pb 8.3-32 mg/kg, and Zn 20-37 mg/kg. Soil pH ranged from 3.1 to 7.3 and organic matter from 4.9 to 7.1 percent. In addition, some macro-elements including P, K, Mg, and Ca were measured. Regression relationships for Pb accumulation were statistically significant only if pH, OM, and Mg were included as contributing factors. Soil pH was a predictive variable for Cd, whereas a combination of Ca and K best predicted the concentration of Cu. However, the low R^2-values for Cd and Cu indicated a poor model fit, while no significant relations were found for Zn. Corp and Morgan (1991) studied *Lumbricus rubellus* in metal-polluted areas in the United Kingdom. Soil Cd concentration range was 0.46-318 mg/kg, Cu 22-818 mg/kg, Pb 91-16700 mg/kg, and Zn 416-183000 mg/kg. Soil pH ranged from 3.5 to 8.1 and organic matter from 4 to 35 percent. No information was given on the type of soil. A good model fit was obtained for Pb and Zn, if pH was used as a predictive factor. An additional contribution of organic matter was found for Cd. Organic matter rather than pH accounted for a significant amount of the variance of Cu in the model.

Ma (1982;1983) studied *A. caliginosa* and *L. rubellus* in six soils ranging from a light sandy soil to a heavy clay-loam in the Netherlands. Ranges of metal concentrations in soil were 0.22-1.55 mg/kg for Cd, 11-133 mg/kg for Cu, 22-253 mg/kg for Pb, and 24-472 mg/kg for Zn. A reasonable model fit was found for Pb and Zn when using pH and cationic exchange capacity as predictive factors in the regression. Organic matter contributed significantly towards the variation in Cd concentration. Remarkably, soil pH did not contribute significantly to the variation in Cu. Further studies were done with *L. rubellus* in sandy soil near a metal smelter in the Netherlands (Ma et al. 1983). Metal levels of Cd were 0.1-5.7 mg/kg, Cu 1-130 mg/kg, Pb 14-430 mg/kg, and Zn 10-1220 mg/kg. Soil pH ranged from 3.5 to 6.1 and organic matter content from 2.2 to 8.6 percent. Soil metal and pH contributed significantly towards the variance of Cd, Pb, and Zn in the earthworms, while organic matter had an additional effect on Pb accumulation. For Cu accumulation, however, a simple linear model with soil Cu as the sole predictive factor gave the best fit.

Morgan and Morgan (1988) studied *L. rubellus* and *Dendrobaena rubidus* from metal-contaminated areas in the United Kingdom. Soil Cd concentrations were 0.1-350 mg/kg, Cu 26-2740 mg/kg, Pb 170-24600 mg/kg, and Zn 160-45000 mg/kg. Soil pH ranged from 4.3 to 7.8 and organic matter from 1 to 27 percent. No information on the type of soil was given. A reasonably good model fit for Cd and Zn was obtained with no predictive variables other than soil metal concentration. However, R^2 remained relatively low in the model for Cu. For Pb, the inclusion of Ca content gave a significant improvement of R^2. Ma et al. (1997) conducted a field study of soil fauna in river floodplains in the Netherlands. The soil consisted of a calcareous silt loam with pH-KCl ranging between 7.1-7.8 and soil organic matter between 2.5-13.7 percent. Concentration ranges in twelve sites were Cd 0.2-4.8 mg/kg, Cu 20-124 mg/kg, Pb 24-228 mg/kg, and Zn 79-980 mg/kg in the 0-20 cm soil layer.

Species differences in metal accumulation capability have been implicated in a number of studies. The accumulation of Cd, Cu and Pb in areas bordering a busy main road has been reported by Marino et al. (1992) to be greater in *Dendrobaena octaedra* and *D. madeirensis* than in *Aporrectodea rosea* and *A. caliginosa*. Accumulation was lowest in *Lumbricus friendi*, but the size of the differences remained relatively small. Endogeic species such as *A. caliginosa* and *Allolobophora chlorotica* accumulated Cd and Pb better than either *Lumbricus rubellus* or *Aporrectodea longa* in an abandoned lead-zinc mine area (Morgan and Morgan 1992). No species-related differences were found in the accumulation of Cu. The anecics contained the highest concentration of Zn. Pizl and Josens (1995) investigated a number of earthworm species in a roadside soil. *A. caliginosa* was the best accumulator of Cd, whereas Pb was better accumulated in *Aporrectodea icteria* than in *Lumbricus* species. For Cu and Zn, however, the results were less consistent. Terhivuo et al. (1994) studied different

Table 2. Studies selected for the model dataset.

Ref	No. soils	No. areas	No. species	Soil parameters and range	Cd	Cu	Pb	Zn
1	4	5	5	pH 5.7-7.4	+		+	
2	7	27	8	pH 4.2-7.2, OM 2.7-15	+	+	+	+
3	2	4	3	pH 4.6-5.9	+	+	+	+
4	?	9	1	pH 3.5-8.1, OM 4.0-35	+	+	+	+
5	1	3	1	pH 6.0-7.2, OM 2.0-6.9	+	+	+	+
6	4	5	2	pH 6.9-7.0, OM 3.3-7.3	+	+	+	+
7	1	1	1	pH 3.5-6.1, OM 2.2-8.6	+	+	+	+
8	6	6	1	pH 4.7-7.1, OM 2.8-14	+	+	+	+
9	1	1	5	pH 5.0-6.8, OM 2.9-6.5	+	+	+	+
10	1	6	6	pH 5.6-6.8	+	+	+	+
11	2	2	6	pH 6.5-6.8	+		+	+

Ref: 1. Andersen (1979); 2. Beyer et al. (1987); 3. Beyer et al. (1982); 4. Corp & Morgan (1991); 5. Czarnowska & Jopkiewicz (1978); 6. Gish & Christensen (1973); 7. Ma (1982); 8. Ma (1983); 9. Morgan & Morgan (1992); 10. Pizl & Josens (1995); 11. Wright & Stringer (1980).

pedo-ecological groups near a lead smelter in Finland. In areas that were heavily contaminated with Pb at a level of about 1500 mg/kg of soil, Pb concentrations in earthworms were greater in *A. caliginosa* than in either *L. rubellus* or *L. castaneus*, the latter two species showing little difference in this respect. However, in soil with a low Pb content of only about 17 mg/kg, no significant differences in accumulation were present between species.

Studies that conformed to the selection criteria cited above were combined to create one single dataset for meta-analysis of model parameters. Sources that have been used for constructing the dataset are summarised in Table 2. Based on the results of the literature survey, a multiple linear regression model was developed which described the relationship between log earthworm metal concentration and five independent variables:

$$\log [Me]_W = a + b \log [Me]_S + c\ pH + d \log [OM] + e\ endogeic + d\ epigeic$$

Correlation analysis did not indicate the presence of multi-collinearity between terms. Equations for best-fit models are given in Table 3. A regression model containing soil metal concentration as a single independent variable was compared with an extended model containing significant terms of pH, organic matter content, and pedo-ecological group. Coefficients in the models were all significant at the 99% confidence level. The results of the meta-analysis showed that soil pH had a considerable negative influence on the earthworm accumulation of Cd and Pb. By contrast, soil pH appeared to be of only minor importance with respect to the accumulation of Cu and Zn in the worms. The addition of organic matter as a predictive parameter in the regression gave a

Table 3. Regression models and coefficients of determination (R^2) relating metal concentration in earthworms (mg/kg) to acid-extractable soil metal concentration (mg/kg), pH, percent soil organic matter (OM), and pedo-ecological group.

Model	R^2	adj.R^2	s.e.
$\log[Cd_W] = 1.39 + 0.556 \log[Cd_S]$	56.1	55.7	0.3167
$\log[Cd_W] = 2.92 + 0.747 \log[Cd_S] - 0.2101\ pH - 0.5336 \log[OM] + 0.156\ endogeic$	78.3	77.6	0.2251
$\log[Cu_W] = 0.776 + 0.327 \log[Cu_S]$	28.7	28.1	0.2596
$\log[Cu_W] = 0.936 + 0.499 \log[Cu_S] - 0.061\ pH - 0.311 \log[OM] + 0.191\ endogeic + 0.275\ epigeic$	45.6	43.4	0.2303
$\log[Pb_W] = 0.626 + 0.556 \log[Pb_S]$	32.7	32.3	0.6613
$\log[Pb_W] = 2.85 + 0.843 \log[Pb_S] - 0.461\ pH - 0.347 \log[OM] + 0.295\ endogeic$	61.7	60.7	0.5038
$\log[Zn_W] = 2.49 + 0.212 \log[Zn_S]$	21.3	20.8	0.2678
$\log[Zn_W] = 2.80 + 0.224 \log[Zn_S] - 0.064\ pH + 0.130\ epigeic$	33.5	32.2	0.2478

further increase of the R^2–value, but only in the model describing the accumulation of Cd, Pb and Cu, and not in that of Zn. Pedo-ecological group could explain a significant proportion of the variability of the body concentration for all four metals investigated. The coefficient of the epigeic term in the model on Cd and Pb was statistically not significant even at the 90% confidence level and the term was therefore removed, leaving only the endogeic term in the equation. Endogeics were found to be more efficient accumulators of Cd and Pb compared to either the epigeics or anecics. This was significant at the 99% confidence level. The accumulation of Cu was better in both endogeics and epigeics as compared to anecics. In the model for Zn, the endogeic term and log [OM] were not significant even at the 90% confidence level. In the simplified model, the epigeics appeared to be better accumulators of Zn than the group of either endogeics or anecics.

The bioaccumulation models proposed in this study were aimed at predicting metal concentrations in earthworms based on a limited number of both routinely and reliably measurable soil parameters that are related to metal concentration and bioavailability. From the results, it appears that a combination of total acid-extractable metal and pH may already explain a considerable part of the variation in metal accumulation in oligochaetes. The prediction can be further improved by adding organic matter as an additional significant contributing factor. This was found to be the case for the accumulation of Cd, Cu, and Pb, but to a much lesser extent for that of Zn. Zinc is an essential metal element and its internal physiological level may to a certain extent be controlled by homeostatic means. Zn occurs in earthworms at a relatively high natural baseline level (Table 4). Homeostatic control may explain the fact that acid-extractable soil Zn concentration and pH accounted for only a small proportion of the variation of Zn in earthworms and OM had no significant contribution whatsoever.

In summary, the present study presents predictive models for Cd, Cu, Pb and Zn that

Table 4. Baseline concentration levels of metals present in whole body tissues of two developmental stages of the earthworm *Lumbricus rubellus* sampled from silt loam soil (Ma 1983). In mg/kg of dry mass.

Life stage	Cd	Cu	Pb	Zn
Juvenile	0.45	25	< 5	336
Adult	13	12	9	639

The juveniles were newly hatched and the adult stage refers to depurated mature clitellated individuals.

can be used to calculate the amount of these metals in the total body tissues of earthworms in contaminated fields. Regarding the variation in metal accumulation between the various pedo-ecological groups, the literature is not very consistent. However, the anecics generally showed a lower tendency to accumulate Cu compared to other pedo-ecological groups. The various field studies seemed to agree on the suggestion that the endogeics are better accumulators of Cd and Pb than either the epigeics or anecics. Possible reasons for such interspecies differences can be either physiological or behavioural or both, but remain as yet unknown. It is important to note that field-sampled earthworms should always concern identified species and their classification according to pedo-ecological group should preferably be known as well. Also the stage of development of the specimens collected should be defined, as metal concentrations may vary considerably according to the stage of development (Table 4). Other important variation sources include the soil depth in the field. Soil sampling depths that have beeen mentioned in the literature vary between 5 and 20 cm. However, soil properties over this depth range, including metal content, often show a strong vertical gradient. Epigeic species, which live close to the soil surface may thus be differently exposed than endogeics that normally inhabit deeper soil layers of up to about 30 cm. Species belonging to the anecic group can reach even greater depths of one metre or more (Bouché 1977). In view of all these shortcomings of the approach, it may be stated that the bioaccumulation models proposed in the present article may be further improved by careful controlling and standardising the many variation sources that are associated with sampling methodology.

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