

Short communication

## Sources of bioavailable trace metals for earthworms from a Zn-, Pb- and Cd-contaminated soil

Thierry Becquer<sup>a,\*</sup>, Jun Dai<sup>b,1</sup>, Cécile Quantin<sup>c</sup>, Patrick Lavelle<sup>d</sup>

<sup>a</sup>UMR 137 Biodiversité et Fonctionnement des Sols, IRD/Embrapa Cerrados, CP 7091, 71619-970 Brasília-DF, Brazil

<sup>b</sup>UMR 137 Biodiversité et Fonctionnement des Sols, IRD/Université de Paris VI, 32 av. Henri Varagnat, 93143 Bondy, France

<sup>c</sup>UMR 8148 IDES, Université Paris Sud XI, Bat. 504, 91405 Orsay Cedex, France

<sup>d</sup>UMR 137 Biodiversité et Fonctionnement des Sols, IRD/Université de Paris VI, 32 av. Henri Varagnat, 93143 Bondy, France

Received 11 February 2004; received in revised form 3 December 2004; accepted 21 January 2005

### Abstract

The sources of bioavailable metals for earthworms were investigated in a Zn-, Pb- and Cd-contaminated soil. Selective sequential extractions (SSE) of metals were performed on soil samples with different amounts of contamination and compared with the body burden concentration of metals in two earthworm species: *Aporrectodea caliginosa* and *Lumbricus rubellus*. The most labile forms (water extractable and exchangeable) of metals were poorly related with metal accumulation by the earthworms, except for Cd, whereas the moderately available forms (acid-soluble, bound to iron oxides and organic matter) were related to the pattern of metal accumulation by earthworms. This indicates that the ingestion of metals bound to soil components is likely to be a more important uptake route than the dermal uptake of dissolved ions for metals entering the body tissue of earthworms.

© 2005 Elsevier Ltd. All rights reserved.

**Keywords:** Trace metals; Earthworm; Contaminated soils; Selective sequential extraction (SSE); Bioavailability

The influence of metal-contaminated soils on earthworm activity and metal bioaccumulation has been reported many times (Morgan and Morgan, 1999 and references therein). Although the total metal content of soil samples is used in many countries as a criterion for soil pollution (Sheppard et al., 1992), it does not provide precise information on the availability of specific elements to biota. As plant roots accumulate elements from the soil solution, the relationship between dissolved metals and plant toxicity is obvious. Numerous reports suggest that non-complexed or 'free ion', metal species in solution are the forms that are available for uptake by biota (Sauvé et al., 1998; Sauvé, 2003). The free metal activity in the soil solution is known to predict reasonably well the toxicity of Pb and Cu for a variety of types of bioassay (Sauvé et al., 1998). However, most bioassays are related to plant yield or microbial processes.

For terrestrial 'soft-bodied organisms' (such as earthworms), the concept of equilibrium partitioning (EqP) presumes a direct relationship between the tissue concentration, taken up through the derma and the free metal ion activity. Although, EqP is able to predict metal bioavailability under laboratory conditions, it is questionable that it does so under field conditions (Peijnenburg and Jager, 2003). According to Lanno et al. (2004), earthworms accumulate metals from soil either through direct dermal contact with chemicals in the soil solution or soil atmosphere, or else by ingestion of bulk soil or specific soil fractions. Therefore, it is not necessarily reliable to use the same toxicity criteria for different organisms. For soil feeders like earthworms other metal phases than just the soluble one could be more reliable to predict metal bioavailability.

Our aim was to determine metal partitioning in specific compartments of the solid phase of soils in order to understand their relationship with trace metal bioaccumulation in two ecologically different species of earthworm. These were the soil-feeding endogeic *Aporrectodea caliginosa* and the mostly litter-feeding epigeic *Lumbricus rubellus* (Bouché, 1972).

\* Corresponding author. Tel: +55 61 388 98 86; fax: +55 61 248 53 78.

E-mail address: becquer@cpac.embrapa.br (T. Becquer).

<sup>1</sup> Present address: South China Agricultural University, 510642, Guangzhou, China.

Table 1  
Selective sequential extraction (SSE) procedure

Soil fraction (abbreviation)	Extractant	Volume	Conditions
1—Water soluble (WAT)	Ultra pure water	10 ml	20 °C, 30 min, end-over-end shaking
2—Exchangeable (EXCH)	100 mM KCl	10 ml	20 °C, 2 h, end-over-end shaking
3—Acido-soluble (ACI)	1 M NaCOOCH <sub>3</sub>	10 ml	20 °C, 4 h, end-over-end shaking
4—Bound to Mn oxides (MNOX)	100 mM NH <sub>2</sub> OH·HCl	10 ml	20 °C, 30 min, end-over-end shaking
5—Bound to amorphous Fe oxides (FEOX1)	200 mM (NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> O <sub>4</sub> ·H <sub>2</sub> O + 200 mM H <sub>2</sub> C <sub>2</sub> O <sub>4</sub> , pH 3	10 ml	20 °C, 4 h, end-over-end shaking in the dark
6—Bound to crystalline Fe oxides (FEOX2)	CB: Na <sub>3</sub> C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> ·2H <sub>2</sub> O (78.4 g l <sup>-1</sup> ) + NaHCO <sub>3</sub> (9.82 g l <sup>-1</sup> ); Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub> , pH 7	50 ml CB 1 g Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub>	80 °C, 15 min in CB and 30 min after adding dithionite, magnetic agitation
7—Oxidizable fraction (associated with organics and sulphurs) (OXID)	(1) 20 mM HNO <sub>3</sub> —35% H <sub>2</sub> O <sub>2</sub> (2) 3.2 M NH <sub>4</sub> COOCH <sub>3</sub> (20% v/v HNO <sub>3</sub> )	(1) 3–5 ml (2) 5 ml	(1) 85 °C, 5 h or 16 h (2) 85 °C, 30'
8—Residual <sup>a</sup> (RES)	Diacid attack		

<sup>a</sup> 100 mg used.

The study site is located in Nord-Pas-de-Calais, northern France. The soil and earthworm samples were collected at three points, with three replicates, along a soil sequence in a pasture contaminated by Zn, Cd and Pb. The samples and earthworms were labelled P3, P6 and P9, P3 being the closest to (2.5 m), P6 intermediate and P9 furthest from (40 m) the contamination source. The soil sequence and the sampling procedures were described by Dai et al. (2004a,b).

The total metal contents in soil and earthworm samples were determined according to Dai et al. (2004a), by ICP-AES (Jobin Yvon 238) after diacid digestion (conc. HNO<sub>3</sub>: HCl; 2:1) of 0.1 g of sample in a microwave oven.

The partitioning of metals among the compartments of the solid phase was investigated indirectly by selective sequential extractions (SSEs). SSEs are used to determine element partitioning in soils and their evolution (Quantin et al., 2002 and references therein). Even so their use has been criticised (i.e. Beckett, 1989), SSEs also provide a unique way to quantitatively assess bioavailable fractions (Peijnenburg and Jager, 2003). Despite the limitations of their use, the SSE are being used increasingly to study metal accumulation in various soil organisms such as collembola (Crommentuijn et al., 1997) and amphipoda (Hendrickx et al., 2004).

The SSEs (Table 1) we used is an eight-step procedure adapted from Leleyter and Probst (1999) and Quantin et al. (2002). The SSEs were performed with 1 g of soil in 50-ml polypropylene tubes with three replicates for each sampling point. After each extraction step, the tubes were centrifuged at 5200 g for 20 min. The supernatants were then filtered through 0.45 µm membranes, and the residues washed with 10 ml of ultra pure water, centrifuged again, and then

the supernatants were pooled. Metals were measured by ICP-AES. The residues were dried at 40 °C before the next extraction step. This extraction procedure provides an operationally defined soil-phase fractionation. Each chemical form was labelled according to the targeted geochemical compartment extracted during each extraction step. These were water soluble (WAT); exchangeable (EXCH); acid-soluble (ACI); bound to Mn oxides (MNOX); bound to amorphous or poorly crystallised Fe oxides (FEOX1); bound to well crystallised Fe oxides (FEOX2); bound to oxidisable compounds (organics and sulphides) (OXID); residual (RES).

Descriptive statistical analyses were made with StatView 4.0 Software (Abacus Concepts, Inc.). Principal Component Analysis (PCA) was performed with ADE-4 Software (Thioulouse et al., 1997) to compare soil metal fractionation with the bioaccumulation of metals by the two earthworm species.

The main characteristics of the soil samples were presented in Dai et al. (2004a,b). Total metals analysis showed a very high content of Cd, Pb and Zn in sample P3 with sharp decrease of their concentrations along the contamination gradient, from P3 to P9 (Table 2). A sharp decrease in earthworm metal content with the decreasing soil metal content was found (Table 3). However, the metal contents of the two earthworm species are not statistically different (with the exception of Cd for the point P3),

Table 2  
Total metal content of the soil samples (mean ± standard deviation, n=3)

Sampling site	Distance to pollution origin (m)	Cd (mg kg <sup>-1</sup> )	Pb (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
P3	2.5	5.0 ± 0	335 ± 41	1030 ± 69
P6	30	3.0 ± 0	52 ± 5.4	176 ± 7
P9	40	2.5 ± 0	31 ± 3.5	108 ± 12

Table 3  
Total metal content of the two earthworm species *Aporrectodea caliginosa* and *Lumbricus rubellus* (mean ± standard deviation, n=3)

Sampling site	Earthworm species	Cd (mg kg <sup>-1</sup> )	Pb (mg kg <sup>-1</sup> )	Zn (mg kg <sup>-1</sup> )
P3	<i>caliginosa</i>	48 ± 30	78 ± 70	1985 ± 422
	<i>L. rubellus</i>	18 ± 4.9	30 ± 8.4	1838 ± 316
P6	<i>A. caliginosa</i>	19 ± 4.2	12 ± 7.6	1090 ± 574
	<i>L. rubellus</i>	17 ± 2.3	6.5 ± 1.1	1053 ± 115
P9	<i>A. caliginosa</i>	21 ± 8.3	3.6 ± 2.2	1321 ± 485
	<i>L. rubellus</i>	11 ± 2.9	1.4 ± 0.8	764 ± 86

Table 4  
Concentrations of Cd, Pb, and Zn in the compartments of the soils after selective sequential extraction

Metal	Sampling site	WAT	EXCH	ACI	MNOX	FEOX1	FEOX2	OXID	RES
Cd	P3	0.09 ± 0.03	0.13 ± 0.01	1.7 ± 0.23	0.08 ± 0	0.67 ± 0.11	1.3 ± 0.58	0.29 ± 0.05	2.0 ± 0
	P6	0.09 ± 0.03	0.05 ± 0.01	0.20 ± 0.20	0.05 ± 0.01	0.33 ± 0.11	0 ± 0	0.07 ± 0.01	1.5 ± 0
	P9	0.03 ± 0.01	0.02 ± 0.02	0.13 ± 0.11	0.04 ± 0	0.40 ± 0	0 ± 0	0.05 ± 0.01	0.97 ± 0.06
Pb	P3	0.43 ± 0.17	0.41 ± 0.06	147 ± 15	0.69 ± 0.10	33 ± 2.6	46 ± 14.8	61 ± 7.2	30 ± 1.6
	P6	0.19 ± 0.10	0.12 ± 0.05	15 ± 3.3	0.05 ± 0.04	13 ± 0.42	4.3 ± 3.5	14 ± 14	16 ± 9.6
	P9	0.13 ± 0.05	0.11 ± 0.11	11 ± 0.35	0.08 ± 0.06	8.2 ± 0.87	3.0 ± 3.0	8.1 ± 6.5	9.0 ± 0.86
Zn	P3	2.90 ± 0.47	19.1 ± 0.67	557 ± 26	27 ± 3.5	285 ± 36	70 ± 4.0	49 ± 17	135 ± 15
	P6	1.23 ± 0.33	4.4 ± 0.84	80 ± 14	8.6 ± 1.4	35 ± 2.1	21 ± 0.58	9.9 ± 1.6	46 ± 8.5
	P9	0.65 ± 0.12	1.8 ± 0.66	61 ± 2.6	6.3 ± 0.55	23 ± 0.95	15 ± 1.0	6.8 ± 0.39	40 ± 9.3

although the two species differ according to their feeding patterns, either litter feeding or soil feeding.

The cumulative amounts of metals recovered during the eight-step SSE procedure (Table 4) were either slightly higher or lower than those obtained by total analysis, particularly for the least contaminated samples. Such differences can be attributed to the efficiency of the simple diacid extraction, which is probably less efficient for metal extraction than after eight extraction steps, which considerably destabilises the soil matrix. Metals were mainly

associated with ACI (8–47%), FEOX1 (9–24%), FEOX2 (0–20%) and RES (8–64%), respectively. From 16 to 18% of Pb were also associated with OXID. The amounts of metals in the most labile fractions (WAT and EXCH) were relatively low except for Cd (3.8% and 2.1%, respectively). In the most polluted soil (P3), the fractions ACI and FEOX2 increased substantially (for Cd and Pb) whereas the RES fraction decreased.

A Principal Component Analysis (PCA, Fig. 1a–c) was performed with the percentage of metal (Zn, Cd, Pb) in each

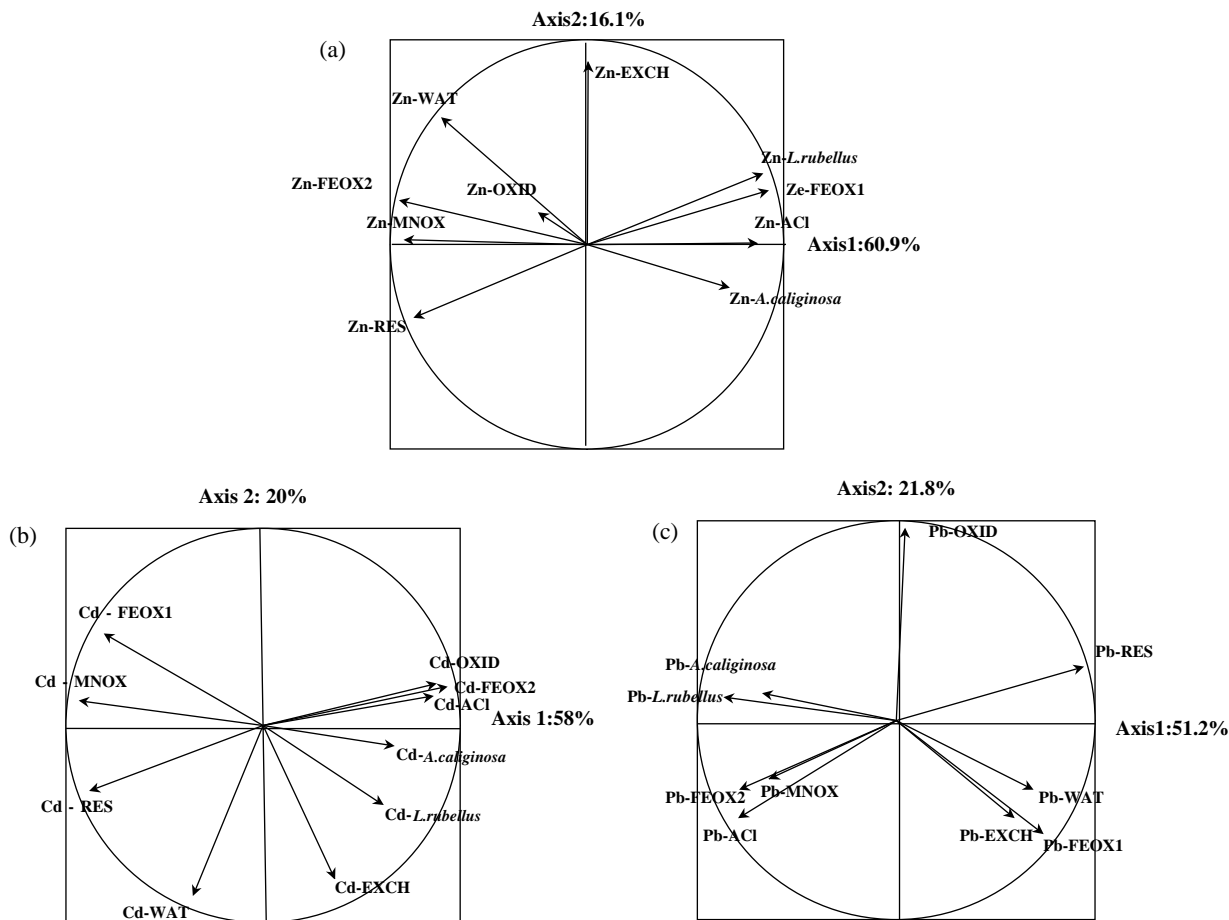


Fig. 1. Correlation circles of metal contents in two earthworm species (*A. caliginosa* and *L. rubellus*) and concentrations of heavy metals in the different soil compartments after selective sequential extractions (SSE) for Zn (a), Cd (b) and Pb (c) by Principal Component Analysis.

fraction of the SSEs and their concentrations in the body burden of the two earthworm species in order to analyse the relationships between these variables. For Zn (Fig. 1a), the first principal component, with a relative inertia of 60.9%, was positively determined by the Zn concentration in the two earthworm species and the fractions ACI and FEOX1, and negatively determined by the fractions RES, MNOX, FEOX2, and OXID. The second principal component, with a relative inertia of 16.1%, was positively determined by the fractions EXCH and, to a lesser extent, WAT. Therefore, the ACI and FEOX1 compartments mainly explain the Zn content of earthworms, whereas the EXCH and WAT fractions were poorly related to metal accumulation by earthworms. The same general trends were observed for Pb (Fig. 1c). For Cd, both the moderately available fractions (ACI, FEOX2 and OXID fractions) and the more easily available EXCH fraction determined the earthworm contents.

The distribution of metals among the soil phases and the solubility of metal-bearing minerals are of great importance for the bioaccumulation by earthworms as the main pathways for chemical absorption are the skin for soluble elements, and gut and digestion for the insoluble ones (Weltje, 1998). Cadmium tends to be more mobile in soils and therefore, more available than many other heavy metals, mostly Pb and Cu (Alloway, 1990). For Cd, the relationships between earthworms bioaccumulation and the soil fractions (Fig. 1b) show that the most available Cd is related to either the exchangeable fraction (Cd-EXCH) or the poorly labile fraction (Cd-OXID, Cd-ACI, Cd-FeOX2). Pb (Fig. 1c) is usually bound to organic matter including that in the soil solution and therefore is not available for dermal uptake. Pb shows an inverse relationship (along the first axis of the PCA) between earthworm bioaccumulation and the most labile fraction of the metal (Pb-WAT, Pb-EXCH). Thus the relationships between metal partitioning evaluated by SSE and metal accumulation in earthworms showed that the most labile forms of metals (WAT, EXCH) are poorly related with metal accumulation by the earthworms. On the other hand the moderately available forms (ACI, FEOX, OXID) better reflected metal accumulation by earthworms. Zn shows an intermediary behaviour between Cd and Pb. These results are fairly consistent with the relative affinity of metals for various soil, generally  $Pb > Zn > Cd$  (Morera et al., 2001). This indicates that the ingestion of metals bound to soil components followed by their cellular assimilation are likely to be the most important pathway for metals to enter the body tissue of invertebrates, whereas dermal uptake of dissolved ions is less important, except perhaps for Cd.

The effects of the specific foods in the diet of either of the two earthworms on metal bioavailability were not demonstrated (except for one value for Cd). The conditions of the display of any difference in the behaviour of the two species in metal accumulation, even though the two species feed on different compounds in soil under natural conditions.

However, metals can be bound on the surfaces of mineral and organic compounds as poorly exchangeable elements. Thus, the two earthworm species can accumulate equivalent amount of metal even though their main source of food differs. In both cases the sources of metals (Pb, Zn) are not the soluble metals (dermal uptake hypothesis), but probably are metals bound to minerals or organic compounds (ingestion uptake).

## Acknowledgements

Financial support for this study was provided by the Institut de Recherche pour le Développement, France. Education Ministry of China had partly financed Jun Dai's stay in France.

## References

- Alloway, B.J., 1990. Cadmium, in: Alloway, B.J. (Ed.), Heavy Metals in Soils. Wiley, New York, pp. 100–124.
- Beckett, P.H.T., 1989. The use of extractants in studies on trace metals in soils, sewage sludges, and sludge-treated soils. *Advances in Soil Science* 9, 143–176.
- Bouché, M.B., 1972. Lombriciens de France—Ecologie et Systématique. INRA, Paris.
- Crommentuijn, T., Doornekamp, A., Van Gestel, C.A.M., 1997. Bioavailability and ecological effects of cadmium on *Folsomia candida* (Willem) in an artificial soil substrate as influenced by pH and organic matter. *Applied Soil Ecology* 5, 261–271.
- Dai, J., Becquer, T., Rouiller, J.H., Reversat, G., Bernhard-Reversat, F., Lavelle, P., 2004a. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils. *Soil Biology & Biochemistry* 36, 91–98.
- Dai, J., Becquer, T., Rouiller, J.H., Reversat, G., Bernhard-Reversat, F., Lavelle, P., 2004b. Influence of heavy metals (zinc, cadmium, lead and copper) to some micro-biological characteristics of soils. *Applied Soil Ecology* 25, 99–109.
- Hendrickx, F., Maelfait, J.-P., Bogaert, N., Tojal, C., Du Laing, G., Tack, F.M.G., Verloo, M.G., 2004. The importance of biological factors affecting trace metal concentration as revealed from accumulation patterns in co-occurring terrestrial invertebrates. *Environmental Pollution* 127, 335–341.
- 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.
- Leleyter, L., Probst, J.-L., 1999. A new sequential extraction procedure for the speciation of particulate trace elements in river sediments. *International Journal of Environmental Analytical Chemistry* 73, 109–128.
- Morera, M.T., Echeverria, J.C., Mazkarian, C., Garrido, J.J., 2001. Isotherms and sequential extraction procedures for evaluating sorption and distribution of heavy metals in soils. *Environmental Pollution* 113, 135–144.
- Morgan, J.E., Morgan, A.J., 1999. The accumulation of metals (Cd, Cu, Pb, Zn and Ca) by two ecologically contrasting earthworm species (*Lumbricus rubellus* and *Aporrectodea caliginosa*): implications for ecotoxicological testing. *Applied Soil Ecology* 13, 9–20.
- Peijnenburg, W.J.G.M., Jager, T., 2003. Monitoring approaches to assess bioaccessibility and bioavailability of metals: matrix issues. *Ecotoxicology and Environmental Safety* 56, 63–77.

- Quantin, C., Becquer, T., Rouiller, J.H., Berthelin, J., 2002. Redistribution of metals in a New Caledonia Ferralsol after microbial weathering. *Soil Science Society of America Journal* 66, 1797–1804.
- Sauvé, S., 2003. The role of chemical speciation in bioavailability, in: Naidu, R., Gupta, V.V.S.R., Kookana, R.S., Rogers, S., Adriano, D. (Eds.), *Bioavailability, Toxicity and Risk Relationships in Ecosystems*. Science Publishers, Enfield, pp. 59–82.
- Sauvé, S., Dumestre, A., McBride, M., Hendershot, W., 1998. Derivation of soil quality criteria using predicted chemical speciation of  $Pb^{2+}$  and  $Cu^{2+}$ . *Environmental Toxicology and Chemistry* 17, 1481–1489.
- Sheppard, S.C., Gaudet, C., Sheppard, M.I., Cureton, P.M., Wong, M.P., 1992. The development of assessment and remediation guidelines for contaminated soils, a review of the science. *Canadian Journal of Soil Science* 72, 359–394.
- Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., 1997. ADE-4: a multivariate analysis and graphical display software. *Statistics and Computing* 7, 75–83.
- Weltje, L., 1998. Mixture toxicity and tissue interactions of Cd, Cu, Pb and Zn in earthworms (Oligochaeta) in laboratory and field soils: a critical evaluation of data. *Chemosphere* 36, 2643–2660.