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SHORT COMMUNICATION

Mathilde I. Zorn · Cornelis A. M. Van Gestel ·
Herman Eijsackers

The effect of *Lumbricus rubellus* and *Lumbricus terrestris* on zinc distribution and availability in artificial soil columns

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Abstract This study investigated the impact of epigeic and (epi)anecic earthworms on the distribution and availability of zinc in the soil profile. Experiments were carried out with *Lumbricus rubellus* and *Lumbricus terrestris* in perspex columns (Ø 10 cm), filled with 20 to 23 cm non-polluted soil [organic matter 2%, clay 2.9%, pH 6.4 (0.01 M $CaCl_2$)], that was covered by a 3- to 5-cm layer of aged zinc-spiked soil (500 mg Zn/kg dry soil) and another 2 cm non-polluted soil on top. After 80 days, columns were sacrificed and sampled in a depth profile. Earthworm casts, deposited on top of the soil, were collected. Each sample was analyzed for total and 0.01 M $CaCl_2$-exchangeable zinc concentrations. *L. rubellus* did not go deeper than 3 cm into the soil and therefore no effect on zinc distribution in the soil could be detected. For *L. terrestris*, total zinc concentrations in the non-polluted layers were slightly but significantly higher in columns with earthworms, and so were the $CaCl_2$-exchangeable zinc concentrations in the polluted layers of these columns. Casts of *L. terrestris* collected from the soil surface showed higher total zinc concentrations than those from non-polluted soil. Casts were mainly placed on top of the soil. This study showed that these epigeic and (epi) anecic species have only a slight effect on zinc availability, and that deep burrowing species, like *L. terrestris*, are able to transport polluted soil from deeper layers to the soil surface.

M. I. Zorn (✉) · C. A. M. Van Gestel · H. Eijsackers
Institute of Ecological Science, Vrije Universiteit,
De Boelelaan 1085,
1081 Amsterdam, The Netherlands
e-mail: mathilde.zorn@ecology.falw.vu.nl
Tel.: +31-20-4447066
Fax: +31-20-4447123

H. Eijsackers
Alterra, Wageningen University and Research Centre,
P.O. Box 47, 6700 Wageningen, The Netherlands

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Introduction

Earthworms play an are important part in soil functioning by influencing decomposition processes, like fragmentation of organic litter (Schulman and Tuinov 1999) and stimulation of microbial activity (Binet et al. 1998), and by bioturbation, increasing soil porosity and water infiltration (Pitkanen and Nuutinen 1998; Lamandé et al. 2003). These actions of earthworms contribute to soil formation (Johnson 2002) and sometimes to indirect effects, such as burying of archaeological artifacts (Armour-Chelu and Andrews 1994), and PCB remediation (Singer et al. 2001; Luepromchai et al. 2002).

Recently new effects of bioturbation on the availability of heavy metals have been found (Zorn et al. 2004). Cheng and Wong (2002) found an increase in zinc availability in a loamy soil after 40 days incubation with an anecic *Pheretima* sp. In contrast, Zorn et al. (2004) found a decrease in zinc availability in a sandy soil after 175 days incubation with endogeic *Aporrectodea caliginosa*, but no effect on zinc availability in the presence of endogeic *Allolobophora chlorotica*. Both these endogeic species transported polluted soil, with *A. caliginosa* tending to move soil upward towards to the soil surface, while *A. chlorotica* mixed it below-ground on a subsurface level.

The effect of a given earthworm species on any of these processes depends highly on its ecology. Epigeic and (epi) anecic species, like *Lumbricus rubellus* and *L. terrestris*, burrow less through the soil than do endogeic species, like *A. caliginosa* (Lavelle and Spain 2001; Jégou et al. 1998). It is unclear, however, if these epigeic and (epi)anecic species also have some influence on heavy metal transport and availability in soil.

The objective of this study was to determine the impact of bioturbation of epigeic and (epi)anecic earthworm species on the distribution and availability of zinc in a soil profile.

Materials and methods

Perspex columns (Ø 10 cm, length 40 cm, *n* =24), with some small holes at the top and bottom to allow air and water flow, were constructed in such a way that they could be split lengthwise. This made it possible to sample soil at specific depths.

Non-polluted (non-spiked) soil and 10-year-aged zinc-spiked soil [approx. 500 mg Zn/kg dry soil (applied as $ZnCl_2$ Merck, rein ≥95% pure)] were collected from an earlier semi-field experiment, carried out by Smit et al. (1997). Both soils, as described by Smit et al., contained 2% organic matter, 2.9% clay and had a pH (0.01 M $CaCl_2$) of approximately 6.4. The soil was sieved, air dried and remoistened to 50% of the maximum water holding capacity, corresponding with a moisture content of 15% dry weight (w/w).

Earthworms (*L. rubellus* and *L. terrestris*) were collected from a grassland soil in the Afferdensche and Deestsche Waarden floodplain, in the central part of the Netherlands, on the south bank of the Waal, a tributary of the river Rhine (longitude 51°54′N, latitude 5°39′E). Both species were maintained in potting compost in a climate room (12°C, 80% RH, 12-h light) until use. Earthworms were placed on wet filter paper, for 24 h before use, to void their guts.

For *L. rubellus*, 12 perspex columns were filled with non-polluted soil to a depth of 20 cm, followed by a polluted layer of 3 cm and then a 2-cm layer of non-polluted soil. Soil density was about 1,400 kg/m^3. Six columns received five *L. rubellus* (three adults, two juveniles, mean 1.14±0.15 g FW per column), and six columns were worm-free controls.

Another set of 12 columns were filled for *L. terrestris*, with non-polluted soil to a depth of 23 cm, followed by a polluted layer of 5 cm and a 2-cm layer of non-polluted soil, resulting in a total soil column length of 30 cm. This increase in depth was used because *L. terrestris* prefers a deeper habitat. Soil density was about 1,400 kg/m^3. Six columns each received one adult *L. terrestris* (mean ± SD 4.13±0.33 g FW), and six columns were worm-free controls.

All columns received approx. 1.75 g wet poplar leaves (*Populus* sp.) on top of the soil at the start of the experiment and again on day 40.

The columns were placed in a climate room (12°C, 80% RH, 12-h light). After 80 days the leaf remains were removed from the soil surface, casts collected, and the soil columns were opened, destructively sampled and soil was taken in 1-cm increments to a depth of 15 cm for the *L. rubellus* columns and to a depth of 10 cm and then at 12, 15, 19, 25 and 30 cm for the *L. terrestris* columns. The earthworms were collected, kept for 20 h on wet filter paper to void their guts, and weighed.

Soil samples and casts were analyzed for moisture content, 0.01 M $CaCl_2$-exchangeable and total zinc concentrations. To determine the $CaCl_2$-exchangeable zinc concentration, 25 ml 0.01 M $CaCl_2$ was added to 5 g wet soil and shaken for 2 h. After precipitation, the samples were centrifuged for 20 min at 10,000×*g*. The supernatant was used for zinc measurements.

Total zinc concentration was determined by digesting 1 g dry soil in a mixture of H_2O, concentrated HNO_3 and concentrated HCl (ratio 1:1:3), using a MARS5 microwave. Quality control was maintained by digesting reference samples (SETOC). The measured zinc concentrations did not deviate more than 10% from the reference value. All zinc extracts were analyzed quantitatively by flame atomic absorption spectrometry (Perkin Elmer 1100B AAS).

Statistical analyses

One-way ANOVA was used to test if zinc concentrations in deeper soil layers (below 10-cm depth) within a soil column were significantly different from each other, and to test for differences between columns with and without earthworms. Student's *t*-test was used to compare zinc concentrations in casts and soil layers with and without earthworms. For that purpose the data from columns with earthworms were combined and compared to the combined data from columns without earthworms.

The construction of the soil columns was such that a sharp boundary was created between polluted and non-polluted soil. It was expected that changes would be gradual and not linear over the soil column. For this reason a logistic model was used to describe the zinc profile, starting from the centre of the polluted layer:

$$Y = \frac{c}{1 + \left(\frac{d}{A}\right)^b} + e$$

where Y is the zinc concentration (mg/kg), c is the maximum zinc concentration in the soil profile (mg/kg), d is the depth (cm), A is the depth (cm) at which Y is $c/2$ which corresponds to the turning point of the logistic curve, b is the slope of the logistic curve at point A and e is the zinc concentration in the non-polluted soil layer (mg/kg).

The values of c, d, a and b were estimated for each column. The total zinc concentration in the non-polluted soil layer (e) was also estimated, but the $CaCl_2$-exchangeable zinc concentration was set at zero, because $CaCl_2$-exchangeable concentrations in the non-polluted soil were below the detection limit. To allow for a comparison between zinc profiles in different columns, the profiles were standardized relative to the turning point in the logistic curve by taking a =1 and $D = d/a$.

Results obtained in columns with the same treatment (with or without earthworms) were grouped, and a generalized likelihood ratio test (Sokal and Rohlf 1969) was applied to compare the profiles of the different treatments. In this way, it was possible to test whether earthworm bioturbation affected zinc concentrations in the polluted or non-polluted soil layers, or the shape (slope) of the zinc profile in the columns.

All statistical analyses were run in Systat 5.2.1 (Systat 1992) on a Macintosh computer.

Results

Lumbricus rubellus

Only 17 out of 30 *L. rubellus* were recovered from the soil columns. Neither *L. rubellus* nor burrows of *L. rubellus* were found below a depth of 3 cm. The top 3-cm soil layers were completely mixed with casts, grubbed soil and leaf fragments. This made it impossible to perform the statistical analyses for *L. rubellus*, and therefore no effects of this species on zinc distribution and availability in the soil could be determined.

Lumbricus terrestris

All *L. terrestris* were recovered at the end of the experiment and were active. Each earthworm had made one burrow to the bottom of the soil column, usually with one or two short side channels. Poplar leaves were drawn into the burrow openings. Most casts were deposited at the soil surface. Total fresh weight of the casts was 10.6–29.8 g per column. Soil moisture content was 15–19% with a vertical gradient and highest moisture contents at greater depths (24–30 cm).

The ANOVA on total zinc concentrations showed no differences within the deeper soil layers (below 8 cm) of a soil column, no differences in the non-polluted soil layers between the columns with earthworms and no differences in the deeper soil layers between the columns without earthworms. The non-polluted soil layers of the columns with earthworms had a significantly higher zinc concentration than those without earthworms (ANOVA; P =0.005; Table 1).

Zinc concentrations in casts were significantly higher than the zinc concentrations in the non-polluted soil layers with earthworms (Student's t-test; P =0.017), as well as in the non-polluted soil layers without earthworms (Student's t-test; P =0.015).

Table 1 Mean total and 0.01 M $CaCl_2$-exchangeable zinc concentrations (± SD) in casts and non-polluted soil layers of columns incubated with (+) and without (−) *Lumbricus terrestris* for 80 days. *Different letters* represent a significant difference (t-test; P <0.05)

Lumbricus terrestris	Matrix	Number	Total zinc (mg/kg dry soil)	$CaCl_2$ zinc (mg/kg dry soil)
+	Casts	6	91.4±40.4 a	1.72±2.29
+	Soil	36	33.5±2.52 b	<0.10
−	Soil	36	31.0±2.76 c	<0.10

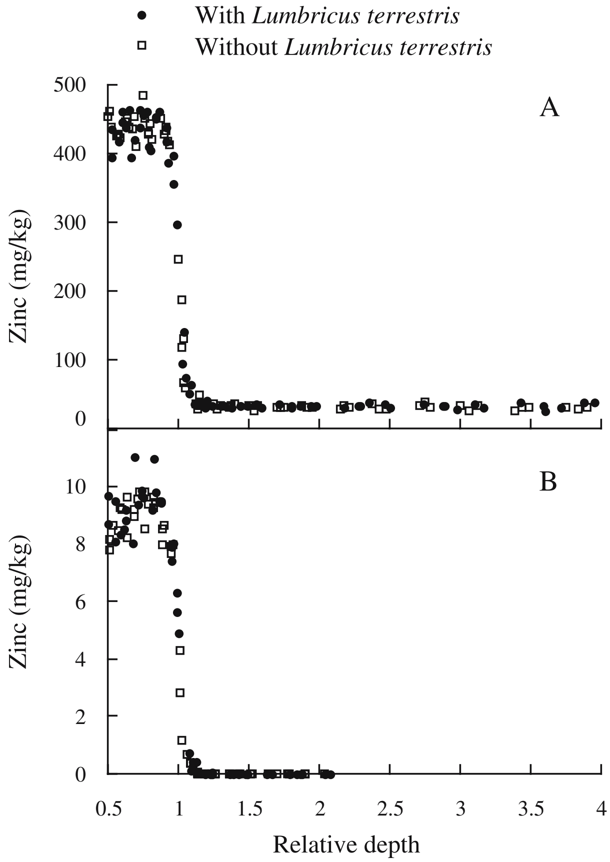

Fig. 1 Total zinc (**a**) and $CaCl_2$-exchangeable zinc (**b**) concentrations at different depths in soil columns incubated for 80 days, with and without *Lumbricus terrestris*. Depth is expressed relative to the centre of the slope between the high and low concentration levels (polluted and non-polluted soil)

Total and $CaCl_2$-exchangeable zinc concentrations are shown in Fig. 1a and b. Profile depth is given relative to the turning point. Corresponding statistical data are given in Table 2. There were no changes in total zinc concentrations between columns with and without *L. terrestris*. The $CaCl_2$-exchangeable zinc concentration in the polluted layers was significant higher (Student's t-test; P = 0.045) in columns with earthworms (mean ± SD, 9.37± 0.81 mg/kg) than in columns without earthworms (mean ± SD, 8.96±0.67 mg/kg).

Table 2 Results of the generalized likelihood ratio test on the zinc profiles in columns incubated for 80 days with and without *Lumbricus terrestris*. Presented are χ^2 values for the differences in c (highest zinc concentration in the columns), b (slope of the curve) and e (lowest zinc concentration). Effects of earthworms are significant when χ^2 > 3.84 at P <0.05, indicated by * (*N.D.* not determined)

	χ^2 Values		
	c	b	e
Total zinc	0.73	1.03	0.37
$CaCl_2$ zinc	6.09*	1.32	N.D.

Discussion

L. rubellus did not go deeper than 3 cm into the soil. These 3 cm consisted of a mixture of casts, grubbed soil and leaf fragments. As an epigeic species it was to be expected that *L. rubellus* would not go deep into the soil (Lavelle and Spain 2001), but in field situations this species is often found deeper, the average depth in a Swedish study being 6 cm (Nordström and Rundgren 1973). Maybe the presence of wet poplar leaves on the columns prevented the top soil from drying out, causing a better habitat for *L. rubellus* than the soil itself and thus preventing the earthworms from burrowing deeper.

Casts of *L. terrestris* contained elevated zinc concentrations, which indicates that this species did not avoid the polluted soil. The slightly but significantly elevated zinc concentrations in the deep, non-polluted layers in the columns containing *L. terrestris* demonstrate that some contaminated material was carried downward. Possibly this comprises compressed casts or mucus, containing elevated zinc levels, due to uptake of metals from the polluted soil layers as previously reported (Binet and Curmi 1992; Jégou et al. 2001). Considering *L. terrestris* as a deep burrowing species, it is possible that the soil brought to the surface by means of surface casting can originate from a depth of 30 cm (this study) or even 3 m (Sims and Gerard 1999).

L. terrestris did increase the availability of zinc, as indicated by the elevated $CaCl_2$-exchangeable zinc concentrations, in the polluted layers. This result is in agreement with Cheng and Wong (2002), who also found an increase of heavy metal availability due to the activity of the anecic *Pheretima* sp. On the other hand, Zorn et al. (2004) found a decrease in $CaCl_2$-exchangeable zinc for the endogeic *A. caliginosa*. However, this effect was observed after 175 days, but not after 80 days, while the present study only lasted for 80 days. This further confirms that earthworm impact on metal availability in soil is a complex issue. Why *L. terrestris* increases zinc availability while *A. caliginosa* decreases zinc availability is unclear. Perhaps it depends on differences in mucus production or composition (Le Bayon and Binet 1999; Schrader 1994).

This study showed that a deep burrowing species, such as *L. terrestris*, can bring polluted soil from deeper layers to the soil surface and may increase metal availability in soil, which can be an important factor in zinc toxicity in ecosystems. The soil brought up to the surface can be redistributed by rainfall, water runoff and flooding (Le Bayon and Binet 1999; 2001). In this way, such earthworms may increase metal exposure of surface-dwelling organisms even over a larger area. Ma et al. (2003) had previously found an increase in metal uptake of up to 53% in plants (*Leucaena leucocephala*) in soils with earthworms (*Pheretima guillelmi*). Still a lot is unknown about metal fluxes through soil, plant and animal interactions.

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References

Armour-Chelu M, Andrews P (1994) Some effects of bioturbation by earthworms (Oligochaeta) on archaeological sites. J Archaeol Sci 21:433–443

Binet F, Curmi P (1992) Structural effects of *Lumbricus terrestris* (Oligochaeta: Lumbricidae) on the soil-organic matter system: micromorphological observations and autoradiographs. Soil Biol Biochem 12:1519–1523

Binet F, Fayolle L, Pussard M (1998) Significance of earthworms in stimulating soil microbial activity. Biol Fertil Soils 27:79–84

Cheng JM, Wong MH (2002) Effects of earthworms on Zn fractionation in soils. Biol Fertil Soils 36:72–78

Jégou D, Cluzeau D, Wolf HJ, Gandon Y, Tréhen P (1998) Assessment of the burrow system of *Lumbricus terrestris*, *Aporrectodea giardi* and *Aporrectodea caliginosa* using X-ray computed tomography. Biol Fertil Soils 26:116–121

Jégou D, Schrader S, Diestel H, Cluzeau D (2001) Morphological, physical and biochemical characteristics of burrow walls formed by earthworms. Appl Soil Ecol 17:165–174

Johnson DL (2002) Darwin would be proud: bioturbation, dynamic denudation, and the power of theory in science. Geoarchaeology 17:7–40

Lamandé M, Hallaire V, Curmi P, Pérès G, Cluzeau D (2003) Changes of pore morphology, infiltration and earthworm community in a loamy soil under different agricultural managements. Catena 54:637–649

Lavelle P, Spain AV (2001) Soil ecology. Kluwer, Dordrecht

Le Bayon RC, Binet F (1999) Rainfall effects on erosion of earthworm casts and phosphorus transfers by water runoff. Biol Fertil Soils 30:7–13

Le Bayon RC, Binet F (2001) Earthworm surface casts affect soil erosion by runoff water and phosphorus transfer in a temperate maize crop. Pedobiologia 45:430–442

Luepromchai E, Singer AC, Yang C-H, Crowley DE (2002) Interactions of earthworms with indigenous and bioaugmented PCB-degrading bacteria. FEMS Microbiol Ecol 41:191–197

Ma Y, Dickinson NM, Wong MH (2003) Interactions between earthworms, trees, soil nutrition and metal mobility in amended Pb/Zn mine tailings from Guangdong, China. Soil Biol Biochem 35:1369–1379

Nordström S, Rundgren S (1973) Associations of lumbricids in southern Sweden. Pedobiologia 13:301–326

Pitkanen J, Nuutinen V (1998) Earthworm contribution to infiltration and surface runoff after 15 years of different soil management. Appl Soil Ecol 9:411–415

Schrader S (1994) Influence of earthworms on the pH conditions of their environment by cutaneous mucus secretion. Zool Anz 233:211–219

Schulman OP, Tuinov AV (1999) Leaf litter fragmentation by the earthworm *Lumbricus terrestris* L. Pedobiologia 43:453–458

Sims RW, Gerard BM (1999) Earthworms. Notes for the identification of British species. Field Studies Council, Shrewsbury

Singer AC, Jury W, Luepromchai E, Yahng C-S, Crowley DE (2001) Contribution of earthworms to PCB bioremediation. Soil Biol Biochem 33:765–776

Smit CE, Van Beelen P, Van Gestel CAM (1997) Development of zinc bioavailability and toxicity for the springtail *Folsomia candida* in an experimentally contaminated field plot. Environ Pollut 98:73–80

Sokal RR, Rohlf FJ (1969) Biometry. The principles and practice of statistics in biological research. Freeman, San Francisco

Systat (1992) SYSTAT 5.2.1. Systat, Evanston, Ill.

Zorn MI, Van Gestel CAM, Eijsackers H (2004) The effect of two endogeic earthworm species on zinc distribution and availability in artificial soil columns. Soil Biol Biochem (in press)