Bull. Environ. Contam. Toxicol. (1991) 47:296–301

Bulletin of Environmental Contamination and Toxicology

Differences in the Accumulated Metal Concentrations in Two Epigeic Earthworm Species (*Lumbricus rubellus* and *Dendrodrilus rubidus*) Living in Contaminated Soils

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Differences have been found in the concentrations of metals accumulated by closely related species of terrestrial invertebrates living in the same contaminated microhabitat : isopods (Hopkin, 1990; Hopkin, Martin and Moss, 1985; Morgan *et al.*, 1986; Morgan and Winters, 1987); slugs (Morgan *et al.*, 1986; Greville and Morgan, 1989; 1990); snails (Withey and Morgan, in preparation); and earthworms (Ireland and Richards, 1977; Morgan and Morris, 1982; Morgan *et al.*, 1986; Morris and Morgan, 1986; Morgan and Morgan, 1988a, 1988b).

Sims and Gerard (1985) described a system for dividing earthworms into three ecophysiological groups : (a) epigeic species (pigmented, litter-feeding, litter inhabiting); (b) endogeic species (non-pigmented, mineral-soil inhabiting); (c) anecic species (deep, vertical burrowing). Observed differences in the tissue metal concentrations of representative epigeic and endogeic species (Morris and Morgan, 1986; Morgan and Morgan, 1988a), although not fully understood, are probably functions of differences in the vertical distribution of the worms within the soil profile, of the metal composition of their selected ingesta, and of their alimentary functions.

Lumbricus rubellus and *Dendrodrilus rubidus* are acid-tolerant epigeic species, which are often the only species inhabiting the poorly vegetated and heavily contaminated soils associated with many abandoned mine sites. Although both species probably consume similar food materials, observations on worms collected from acidic (Ireland and Richards, 1977) and calcareous (Morgan and Morris, 1982) mine sites indicate that they accumulate significantly different metal concentrations in their tissues : the larger *L. rubellus* accumulates more Zn and Ca, but less Pb and Cd than *D. rubidus*. The aim of the present study was to analyse these two epigeic species sampled from ten diverse sites (one reference and nine contaminated) to determine whether the inter-species differences in relative metal accumulation is a general feature of these sympatrics.

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MATERIALS AND METHODS

The locations of the sampling sites in England and Wales are presented in Table 1. Soils and earthworms were sampled and processed for atomic absorption analysis of Pb, Cd, Zn and Ca as described previously (Morgan and Morgan, 1988b). Differences between the mean metal concentrations in *L. rubellus* and *D. rubidus* at each site were statistically analysed by the Mann-Whitney non-parametric 'U' test.

Table 1: Total Pb, Zn, Cd and Ca concentrations (µg/g dry weight; mean ± S.E.) in the soils.

SITE	Pb	Zn	Cd	Ca
Dinas Powys [ST 146723]	170 ± 7	190 ± 4	0.9 ± 0.01	1790 ± 50
Llantrisant [ST 048822]	6930 ± 170	2030 ± 90	19 ± 1.00	5080 ± 185
Draethen 'M' [ST 217877]	5330 ± 85	16370 ± 720	350 ± 8.00	26420 ± 1320
Draethen 'F' [ST 207870]	16590 ± 290	5470 ± 90	90 ± 1.00	58120 ± 3020
Snailbeach [SJ 374023]	10050 ± 335	6910 ± 350	80 ± 11.00	48920 ± 2170
Minera-1 [SJ 262518]	24550 ± 520	44970 ± 1390	280 ± 20.00	91850 ± 2490
Minera-2 [SJ 226251]	5090 ± 150	12410 ± 560	50 ± 7.00	69440 ± 1940
Cwm Rheidol [SN 732784]	220 ± 3	210 ± 4	0.84 ± 0.02	6780 ± 250
Cwmystwyth [SN 803748]	850 ± 50	160 ± 3	0.09 ± 0.01	170 ± 8
Wemyss [SN 719745]	12370 ± 280	690 ± 50	1.21 ± 0.01	1390 ± 45

number of replicates = 5. Data in parentheses = Ordnance Survey Map References

RESULTS AND DISCUSSION

The measured soil metal concentrations (conc. HNO_3 - extractable fraction) are presented in Table 1, and the whole-worm tissue metal concentrations in Table 2. With but minor exceptions it was found that the tissues of *L. rubellus* contained higher Zn and Ca concentrations, and lower Pb and Cd concentrations than the tissues of *D. rubidus* sampled from a series of diverse contaminated 'soils'; in the majority of cases the differences were statistically significant (Table 2), and confirm some of our provisional observations during an earlier biomonitoring survey (Morgan and Morgan, 1988b). The mean relative metal concentrations in the tissues of *D. rubidus* compared to *L. rubidus* were: Pb = 270% (range 125-540%); Zn = 60% (29-100%); Cd = 190% (71-331%);

Table 2: Comparisons between the whole-body tissue metal (Pb, Zn, Cd, Ca) concentrations (µg/g dry weight; mean ± S.E.) in *Lumbricus rubellus* (*L.r.*) and *Dendrodrilus rubidus* (*D.r.*)

Site	Species	Pb	Zn	Cd	Ca
Dinas Powys	*L.r.* (12) *D.r.* (9)	4 ± 1 12 ± 2 **	390 ± 35 310 ± 40 (N.S.)	14 ± 2 30 ± 4 ****	3200 ± 180 4320 ± 300 **
Llantrisant	*L.r.* (11) *D.r.* (9)	780 ± 100 1340 ± 160 **	1300 ± 90 765 ± 80 ***	130 ± 20 430 ± 60 ****	5040 ± 350 3960 ± 430 (N.S.)
Draethen M	*L.r.* (8) *D.r.* (18)	630 ± 80 3380 ± 230 ****	3110 ± 280 1680 ± 100 ****	580 ± 80 1790 ± 90 ****	11070 ± 850 4550 ± 280 ****
Draethen F	*L.r.* (17) *D.r.* (12)	700 ± 100 2620 ± 390 ****	2360 ± 250 870 ± 120 ****	180 ± 20 410 ± 30 ****	9910 ± 680 3930 ± 370 ****
Minera-1	*L.r.* (11) *D.r.* (16)	2150 ± 350 3080 ± 240 (N.S.)	3870 ± 795 1130 ± 130 ****	160 ± 50 190 ± 10 (N.S.)	10400 ± 1530 3850 ± 130 ****
Minera-2	*L.r.* (8) *D.r.* (12)	1240 ± 230 3070 ± 420 ****	1300 ± 220 1110 ± 130 (N.S.)	120 ± 20 270 ± 30 ***	6110 ± 830 3570 ± 260 ***
Snailbeach	*L.r.* (7) *D.r.* (8)	990 ± 140 2050 ± 450 *	1500 ± 220 690 ± 60 ***	220 ± 20 320 ± 40 (N.S.)	5600 ± 490 3020 ± 300 ***
Cwm Rheidol	*L.r.* (13) *D.r.* (19)	14 ± 2 40 ± 5 ****	610 ± 60 470 ± 20 **	14 ± 2 17 ± 2 (N.S.)	7370 ± 520 3290 ± 170 ****
Cwmystwyth	*L.r.* (15) *D.r.* (24)	2580 ± 350 7780 ± 856 ****	460 ± 40 460 ± 30 (N.S.)	8 ± 1 10 ± 1 **	6660 ± 320 5340 ± 130 ****
Wemyss	*L.r.* (13) *D.r.* (42)	10410±1210 13042± 855 (N.S.)	1140 ± 140 490 ± 20 ****	17 ± 2 12 ± 1 **	7220 ± 400 2820 ± 70 ****

Numbers in parentheses = number of observations; N.S. = not significant at the P<0.05 level; *=P<0.005; ** = P<0.02; *** = P<0.005; **** = P<0.001.

Ca = 53% (37-80%). The present study extended and confirmed the earlier observations (Ireland and Richards, 1977; Morgan and Morris, 1982) on the relative metal accumulation by the sympatric earthworms, *L. rubellus* and *D. rubidus* : the larger epigeic species (*L. rubellus*) accumulated higher concentrations of the essential metals, Zn and Ca, but lower concentrations of the non-essential metals, Pb and Cd, than the smaller epigeic worm (*D. rubidus*). The differences were usually, but not invariably, statistically significant. The occasional site-to-site inconsistencies in the relative accumulation of a given metal by the two species could be attributable to one of at least two factors. First, edaphic interactions (Ma, 1982; Ma *et al.*, 1983; Morgan and Morgan, 1988a, 1988b) may differentially affect the availability of a metal to one species compared to the other. However, it is noteworthy that both species displayed enhanced tissue Pb accumulation from relatively Ca-poor soils (ratios of tissue [Pb] to soil [Pb]: *D.r. at* Cwmystwyth = 9.10; *D.r.* at Wemyss = 1.03; *L.r.* at Cwmystwyth = 3.02; *L.r.*at Wemyss = 0.84) compared to relatively calcareous soils (mean ratios for the other metalliferous sites: *D.r.* = 0.30; *L.r* = 0.10). Second, a local ecotype of one species may be relatively more or less well adapted to withstand metal exposure. Enhanced metal tolerance can be phenotypically expressed in different invertebrate groups, but not necessarily between different populations within a species, either as an increased or a decreased metal-storage capacity (Klerks and Weis, 1987). Recently, a few studies have shown evidence of differentiation between populations of invertebrates, but alas not earthworms, exposed to terrestrial metal pollution : isopods (Van Capelleveen, 1987), snails (Beeby and Richardson, 1987), springtails (Van Straalen *et al.*, 1987; Posthuma, 1990). Posthuma (1990), examining the toxic effects of Zn and Cd on first generation springtails reared in the laboratory, showed that the differentiation was genetic, that the population responses to the two metals was different, and that tolerance was most pronounced in the offspring from populations inhabiting long-established, heavily contaminated sites. Similar studies are required for earthworms.

Our present knowledge of the detailed ecophysiological characteristics of *L. rubellus* and *D. rubidus* is too sparse to offer an explanation of observed differences in metal accumulation. The two species are typically epigeic because they possess actively mineralizing calciferous glands; however, the gland of *L. rubellus* is anatomically more elaborate than the gland of *D. rubidus* (Piearce, 1972; Morgan, 1982). Whether this difference in Ca physiology affects the metabolism of other metals, directly or indirectly, is unknown.

Piearce (1978) showed, from a detailed examination of earthworm gut contents, that different species are much more discriminating consumers of soil/litter constituents than was previously appreciated. Even species consuming similar materials were shown to select different sized particles. The much smaller *D. rubidus* probably selects smaller particulates than *L. rubellus*. Small particles have relatively large surface areas, thus increasing the potential solubility of their bound metals in the earthworm gut. Furthermore, small particles may represent litter of a more advanced state of decomposition, and may be relatively heavily colonized by soil microflora. A number of workers have shown that fungal hyphae can accumulate high concentrations of metals, and may serve as an enriched source of metals for the invertebrates that consume them (see Hopkin, 1990). A better understanding of the food preferences of different earthworm species is urgently required. Finally, the importance of recognising interspecies and inter-population differences in accumulated tissue metal burdens in the context of pollution biomonitoring is self-evident. But the importance of such differences in the evaluation and interpretation of metal-transport pathways within local habitats is only beginning to be appreciated (Hopkin, 1990).

Acknowledgments. We thank Mrs Annette Robson for typing the manuscript, and the Natural Environment Research Council for awarding a studentship to J.E.M.

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Received December 6,1990; accepted January 10, 1991