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Calcium-Lead Interactions in Earthworms: Observations on *Lumbricus terrestris* L. Sampled from a Calcareous Abandoned Leadmine Site

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The accumulation of heavy metals has been measured in earthworms sampled from soils contaminated by: (a) atmospheric pollutants from various anthropogenic sources; (b) sludge application; (c) Pb- and Zn-rich spoil heaps associated with disused metalliferous mines (Ireland 1983). Lead is the metal most intensively studied, and the available evidence suggests that its accumulation by earthworms is influenced by exogenous (soil) and endogenous (tissue) Ca. Although the published literature is limited, it emerges that Pb-Ca interactions may be manifest at three distinguishable ecological/physiological levels. (1) Soil Ca tends to suppress Pb accumulation (Andersen 1979; Ireland 1979; Andersen and Laursen 1982). (2) Observed species differences in Pb burdens may reflect fundamental differences in the Ca metabolism of the species involved (Ireland and Richards 1977; Andersen and Laursen 1982; Morgan and Morris 1982). Whilst this conclusion is in accord with the well-recognised inhibition of Pb transport by Ca in vertebrates, the experimental supportive evidence for earthworms is weak. (3) Tissue Pb accumulation results in an apparent parallel increase in tissue [Ca] (Ireland 1975; Andersen 1979). Again the evidence is fairly limited, and rests on Ireland's (1975) observations on the earthworm Dendrobaena rubida sampled from a heavily Pb-polluted, calcium deficient soil with low pH.

The main purpose of the present study was to determine whether a positive Ca-Pb relationship exists in the tissues of L.terrestris. This species possesses well-developed Ca secretory/excretory glands (Morgan 1981), and may thus be able to homeostatically regulate tissue [Ca]. The worms were sampled from six different 'stations' across a heavily polluted disused Pb/Zn mine site, where the inter-station [Ca] varied by as much as a factor of x10. This heterogeneous site, therefore, offered a good opportunity to study additional aspects of Ca-Pb interactions in an earthworm population under field conditions.

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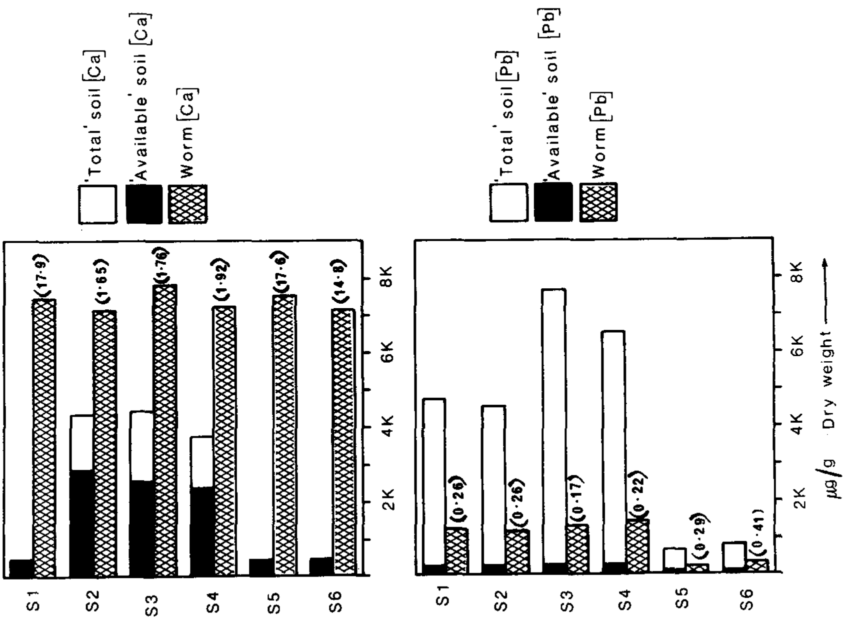

Figure 1. Comparison of the whole-body [Ca] and [Pb] in L.terrestris with soil [Ca] and [Pb] ('total' and 'available'). Mean values only are plotted (see Table 2 for S.E. and n values). Values in parentheses are concentration factors, i.e. worm [Ca]÷ soil [Ca]$_{total}$ and worm [Pb]÷soil [Pb]$_{total}$.

MATERIALS AND MATERIALS

L.terrestris specimens were collected in November 1980 by formalin extraction at a site known as Park Mine (Grid Ref. ST 047823) near Llantrisant, S.Wales. The workings of this mine, besides being in Triassic rocks, extended into Carboniferous limestone which overlies coal measures; lead sulphide (galena) and associated zinc sulphide (sphaelerite) were the main ores present. Work at the site was probably abandoned about 100 years ago, and the whole site has been completely covered by pasture across which runs a narrow strip of mixed beech, holly and elder woodland. Samples were taken at six stations situated on a longitudinal transect across the site: sampling stations 1 and 2 (S1, S2) - 'upper' pasture; S3 and S4 - woodland; S5 and S6 - 'lower' (sloping) pasture.

Extracted worms were immediately washed in clean tap water for 2 minutes, transported to the laboratory, and their gut contents cleaned by maintenance on moistened filter paper (Whatman No.1) in a dark constant-temperature (10°C) room for 4 days. Filter paper was changed daily. The worms consumed the filter paper and were excreting clean paper 'casts' by the fourth day. Soils (0-10 cm depth samples) and worms were prepared for atomic absorption spectrophotometry (Pye/Unicam SP 2900) by a wet-digestion procedure as previously described (Morgan and Morris

1982). 'Available' soil metal concentrations were estimated by extraction in 0.5% (v/v) acetic acid. Data was expressed as Mean ± S.E. Differences between means were assessed wherever appropriate by a 2-tailed Student's t-test.

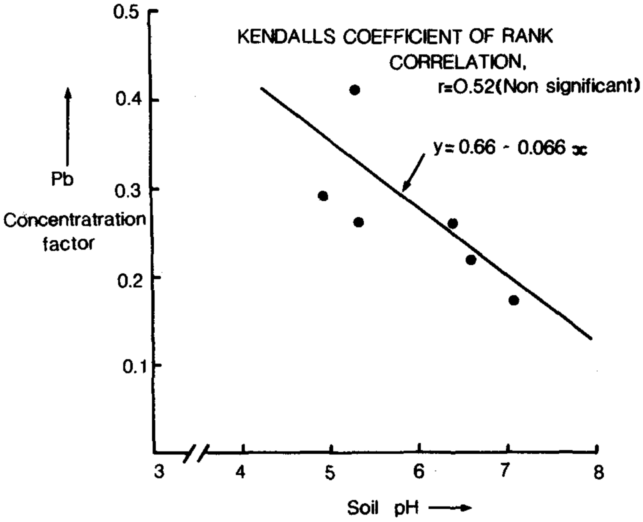

Figure 2. Plot of mean Pb concentration factors in <u>L.terrestris</u> against soil pH at the six sampling stations. The significance of the relationship between the two parameters was determined by Kendall's Rank correlation (r).

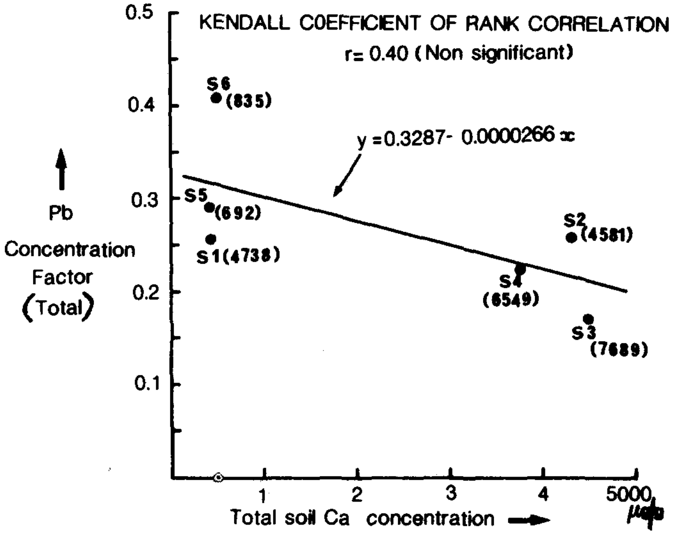

Figure 3. Plot of mean Pb concentration factors in <u>L.terrestris</u> against the mean soil [Ca]$_{total}$. Values in parentheses are the appropriate mean [Pb]$_{total}$ at each station. Note that the correlation (Kendall's r) is poorer than that between Pb concentration factor and soil pH (Figure 2).

Table 1. Metal concentrations (ug/g dry weight) in soils*

		STATION NUMBER					
		S1	S2	S3	S4	S5	S6
[Pb]	Total	4738 ±499	4581 ±468	7689 ±351	6549 ±298	692 ±67	835 ±76
	Extractable	227 ±7	228 ±16	258 ±37	256 ±48	102 ±4	105 ±4
	% **	4.8	5.0	3.4	3.9	14.7	12.6
[Ca]	Total	417 ±71	4329 ±576	4460 ±682	369 ±1022	428 ±28	480 ±49
	Extractable	377 ±64	2854 ±379	2526 ±203	2378 ±48	388 ±24	434 ±44
	% **	90.4	65.9	56.6	63.1	90.7	90.4
	pH ***	5.3	6.4	7.1	6.6	4.9	5.3

* 6 replicates in each case

** $$\% = \frac{\text{mean amount extracted by 5\% acetic acid}}{\text{mean total (i.e. analysed in HNO}_3\text{ digest)}} \times 100\%$$

*** pH measured in 2:1 dry soil:deionized water slurry stirred for 1 hr

Table 2. Metal concentrations (ug/g dry weight) in L.terrestris

	STATION NUMBER					
	S1	S2	S3	S4	S5	S6
[Pb]	1221 ±91 (30)	1191 ±52 (10)	1312 ±134 (10)	1459 ±241 (10)	201 ±16 (10)	338 ±39 (12)
[Ca]	7467 ±168 (30)	7143 ±216 (10)	7840 ±337 (10)	7237 ±300 (10)	7563 ±351 (10)	7130 ±253 (12)

Numbers in parentheses = numbers of observations

RESULTS AND DISCUSSION

The analytical data for soils and L.terrestris are summarized in Tables 1 and 2, and graphically compared in Figure 1. The geochemistry of the site is a highly variable mosaic with soil $[Ca]_{total}$ ranging from 417 ug/g (S5) to 7689 ug/g (S3); and pH ranging from 4.9 (S5) to 7.1 (S3). The 0.5% acetic acid 'available' fractions of both Ca and Pb appear to be pH dependent (Table 1).

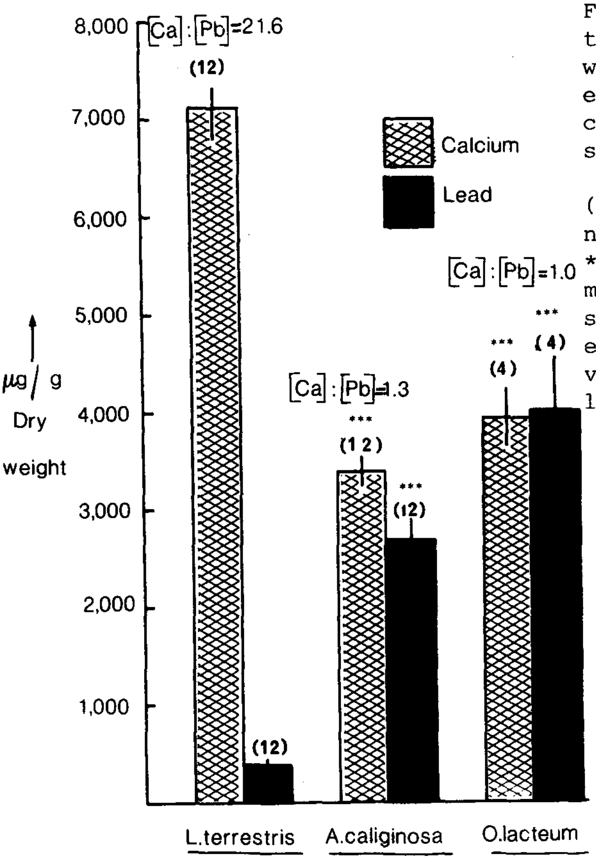

Figure 4. Comparison of the [Ca] and [Pb] in the whole-bodies of 3 earthworm species collected at sampling station S6.

(Numbers in parentheses = number of observations. *** signifies that a given mean value differs significantly from the equivalent L.terrestris value at the $P<0.001$ level).

A striking feature of these findings is that the [Ca] in L.terrestris tissues is maintained remarkably constant (Table 2; Figure 1), with no significant differences ($P > 0.05$) despite the 10-fold variation in soil $[Ca]_{total}$ across the site. In contrast, worm [Pb] generally reflected (albeit imprecisely) the soil $[Pb]_{total}$ (Figure 1). Although Roberts and Johnson (1978) recorded a significant positive correlation between soil [Pb] and the [Pb] in L.terrestris, it is evident that specific soil physicochemical properties also affect the amount of Pb accumulated by terrestrial organisms. For example, we found (Figure 2) that for a given soil [Pb] the worm [Pb] increased as soil pH decreased. Ma (1982), in a very detailed study of Allolobophora caliginosa, demonstrated that worm Pb burdens are determined primarily by soil [Pb] and soil pH, and also but to a lesser extent by the cation exchange capacity of soil. Figures 2 and 3 show that the Pb concentration factor (i.e. worm [Pb]: soil $[Pb]_{total}$) did not exceed 0.41 (S6) at any one of the sampling stations. There is no evidence to suggest that a positive correlation exists between tissue [Pb] and [Ca] in this population of *L.terrestris*. Indeed, these worms are capable of regulating their endogenous [Ca] within fairly narrow limits, and quite independently of large variations in substrate $[Ca]^{total} \pm [Pb]^{total}$ and also in whole-body [Pb].

The present observations contradict the positive correlation between [Pb] and [Ca] in worm tissue reported by Andersen (1979) and Andersen and Laursen (1982). These authors analyzed the earth-worms, *L.terrestris*, from a polluted road-side soil, and various *Allolobophora* species from sludge-ammended soils of fairly neutral pHs. However, their conclusions must be seriously questioned because they plotted together the analytical data for the different species derived from distinctly different sampling sites. Since *L.terrestris* has a significantly higher [Ca] than certain *Allolobophora* species (Figure 4; see also Piearce 1972), and since it was also sampled from an environment more heavily polluted with Pb than that from which *Allolobophora* was derived, the pooling of analytical data for the different species introduces an obvious bias in favour of a positive [Ca]-[Pb] correlation and cannot, therefore, be justified.

The definite positive correlation between tissue [Ca] and [Pb] recorded by Ireland (1975) in *D.rubida* 'transplanted' from relatively Pb-free soil to highly contaminated *and* acidic soil is more readily interpreted and reconciled with the present findings. The Pb concentration factors observed in *D.rubida* (Ireland 1975) and *L.rubellus* living in acidic soil are exceptionally high (2.4 and 2.7, respectively - see Ireland 1983, for other published values). The major subcellular compartment for Pb accumulation/ detoxification in earthworms are the chloragosome granules in the chloragog tissue (Ireland and Richards 1977; Morgan and Morris 1982). We propose that when soil [Pb] is high and pH is low (resulting in high concentration factors) the chloragosome compartment eventually saturates. We further envisage that 'excess' Pb 'floods' into the chloragocytic cytoplasm, and may also be distributed at disproportionately high concentrations in other tissues and cells. If this occurs, then generalized cell-membrane damage would ensue, with a consequent influx of Ca^{2+} from extra-cellular fluids. Presumably the tissue [Pb] in *L.terrestris*, even at the most heavily contaminated stations (S3, S4) at Park mine (which also possessed the highest soil pHs), did not exceed the hypothetical Pb saturation level of the chloragosomes.

When the mean Pb concentration factors were plotted against mean $[Ca]^{total}$ (Figure 3) there was a suggestion of a negative correlation. This could be interpreted to show that high soil [Ca] inhibits Pb accumlulation by worms. Concentration factors must, however, be used and interpreted with some caution (Ma 1982; Ireland 1983). It is salutory that the highest Pb concentration factors (S5=0.29 and S6=0.41) were recorded at the stations with the lowest soil [Pb], and the lowest concentration factors (S3=0.17 and S4=0.22) at the stations with the highest soil [Pb]. Ireland (1983) drew attention to two separate studies where 'anomalous' concentration factors >1 were observed. In both instances soil [Pb] was low. The present study cannot, therefore, contribute to the discussion of the competitive inhibitory role of soil Ca on Pb accumulation. A much more extensive systematic study, designed to separate the intimately-linked effects of soil [Ca] and pH for example (compare Figures 2 and 3), would be required to definitively answer this question.

In addition to L.terrestris, two other earthworm species (A.caliginosa and Octolasion lacteum) were collected from S6 (Figure 4). Unlike L.terrestris, these worms are non-pigmented and they possess non-secretory calciferous glands (Piearce 1972). Less Ca is transported across the intestine of A.caliginosa compared to another Lumbricus species i.e. rubellus (Piearce 1972). The present analytical data clearly show that whole-worm [Pb] is significantly higher in A.caliginosa and O.lacteum compared with L.terrestris (Figure 4). It is tempting to suggest that these differences are related to fundamental differences in the Ca metabolism of these worms; note that both the [Ca] and the [Ca]:[Pb] ratio are significantly higher in L.terrestris than in the other two species. Ecological differences, for example in food selection (Piearce 1978) and other resource and habitat partitioning strategies, cannot however be dismissed as minor influences on the relative bioavailabilities of specific metals to individual species. These and other ecophysiological factors are currently being investigated in our laboratory.

This study therefore confirms that biotic (endogenous) and abiotic (exogenous, geochemical) modulating interactions must receive serious attention in all studies that seek to use earthworms as biomonitors of Pb pollution and its consequences. This general statement can, of course, be extrapolated to include other metals and other organisms in different ecosystems.

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