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Calcium-Lead Interactions Involving Earthworms: An Hypothesis

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This paper surveys the published literature on Ca-Pb interactions involving earthworms, and attempts to rationalize the information within a simple hypothetical scheme. The scheme recognises that Ca-Pb interactions operate within at least 3 major 'compartments': the geochemical or **soil** compartment; a metabolic or transport compartment; and in the cumulative or detoxification (chloragosome) compartment. The scheme also recognises that the consequences of Ca-Pb interactions differ under acidic and calcareous soil conditions. Finally, attention is drawn to those biotic and abiotic areas that require further study if earthworms are to be considered seriously for pollution monitoring purposes.

INTRODUCTION

The direct measurement of heavy metal concentrations in environmental samples such as sediments, water, soil does not necessarily provide an accurate predictive indication of the potential biological significance of the pollutant(s). Total metal-concentration measurements are frequently misleading, because the amount of a metal entering the biomass is determined by specific biotic and abiotic phenomena. Amongst the physico-chemical determinants of metal speciation and bioavailability in different aquatic ecosystems, the pH, temperature, salinity, hardness, and complex-forming organic composition are predominant (Prosi, 1981). Terrestrial ecosystems have not been as thoroughly studied; nevertheless, it is known that similar processes operate (Davies, 1980).

It has been proposed by several workers that a more meaningful

assessment of the impact of metal pollution may be obtained by measuring metal concentrations in selected species of the resident biota. Some of the stated (Phillips, 1980) advantages of this approach are: (i) analytical problems may be alleviated because some organisms accumulate certain heavy metals, **so** that their total body concentrations considerably exceed environmental levels; (ii) the analysis of organisms yields information about the bioavailability of metals as opposed to their total environmental concentrations; (iii) ideally, the organism serves as a biotic integrator of metal pollution over a period of time, thus minimizing the problems associated with transient stochastic fluxes in environmental contamination.

Earthworms have been shown to accumulate heavy metals from a range of anthropogenically polluted situations (see Beyer, 1981, and Ireland, 1983 for references). Indeed, with the possible exception of isopods, earthworms probably accumulate higher metal burdens than other soil animals (Beyer, 1985; Martin and Coughtrey, 1976). Worms have not, however, been used for pollution monitoring purposes, although they clearly possess several qualities that are desirable in organisms chosen for this purpose. They are relatively large and therefore easily analyzed; they are widely distributed but fairly immobile; they are easily identified; they are detritivores, and therefore represent an important step in the transference of metals to higher trophic levels; they sample large volumes of the substrates in which they live, and are thus the terrestrial equivalents of aquatic filter-feeders. In addition to these general features, it is necessary that a simple correlation exists between the metal concentration in the indicator species and in its environment.

Although it has been demonstrated that earthworm lead burdens do increase as soil lead concentration increases (Roberts and Johnson, 1978; Ma, 1982; Morgan, 1985), the relationship may not be a simple one due to the effects of various biotic and abiotic interactions. For example, one factor that directly and indirectly modulates the uptake and accumulation of lead by earthworms is exogenous (soil) and endogenous (metabolic) calcium (Morris and Morgan, 1985). This is not unexpected since lead is known to enter calcium metabolic pathways in vertebrates (e.g. Clarke and Clarke, 1975; Finley and Dieter, 1978).

The purpose of this short paper is to bring together the published

information on calcium-lead interactions involving earthworms within the framework of a hypothetical scheme. Whilst it is recognised that this scheme is probably a gross understatement, it serves to demonstrate some of the complexities of the interrelationships between a toxic heavy metal and a single (albeit important) essential element. Furthermore it may serve two other functions: firstly, to remove some of the ambiguities that exist in the literature; secondly, a heuristic function leading to to critical evaluation of earthworms as bioindicators of lead (and perhaps other metals) pollution in terrestrial ecosystems.

THE HYPOTHETICAL SCHEME

This scheme proposes that Ca-Pb interactions operate within at least three distinguishable eco-physiological compartments: (i) the geochemical or soil compartment; (ii) the metabolic or transport compartment; (iii) the chloragosomal **Pb-accumulating/detoxifying** compartment. Of course these compartments are not necessarily isolated. For example, there is evidence suggesting that the presence of *Dendrobaena rubida* increases the bioavailabilities of Pb, Zn and Ca in contaminated soils (Ireland, 1975a), although this may not be the case for Eisenia *foetida* living in sludge (Neuhauser and Hartenstein, 1980). **A** second aspect of the scheme is that it proposes significant differences in Ca-Pb interrelationships under acidic and calcareous soil conditions; a difference reflected in the relative Ca and Pb concentrations in whole earthworms. For this reason the scheme (Figure 1) is diagrammatically presented in a split form.

A. Calcareous soils (Figure la)

Providing a site contains a sufficient depth of soil and adequate nutrient and moisture content, a wide range of earthworm species may inhabit heavily Pb-polluted calcareous soils (Morgan and Morgan, in prep.). In other words there is no evidence currently available to suggest that metal pollution per **se** is responsible for selectively limiting the distribution of individual species.

High soil Ca concentrations are correlated with lowered Pb

accumulation by earthworms (Andersen, **1979;** Ireland, **1979;** Andersen and Laursen, **1982). A** similar reduction in Pb bioavailability is found in hard-water systems (Prosi, **1981).** The difficulty of dissecting the direct Pb-immobilization role of calcium salts from the concomitant (and perhaps predominant) effect of environmental **pH** or Pb solubility, and thus Pb-bioavailability, must be acknowledged (Morgan, 1985). In addition, it may also be difficult to differentiate between physicochemical and physiological or biochemical metal-interactions; for example, the reduced Pb uptake by rats fed **on** a high-Ca diet (Baltrop and Khoo, **1976)** may be at least partially due to Pb immobilization within the intestinal lumen, and not entirely due **to** transport processes. However, in earthworms it is possible to conclude that the observed Pb 'concentration factor' (i.e. the ratio of worm Pb concentration: 'total' soil-Pb concentration) is invariably lower than unity in calcareous, heavily polluted soils (Ireland, **1983;** Morris and Morgan, **1985).**

The calcium metabolism characteristics of different species may influence the amount of Pb accumulated by them. Earthworms are broadly divisible into two heterogeneous eco-physiological groups: (i) pigmented litter-feeders with active calcium secreting/excreting calciferous glands; (ii) non-pigmented soil consumers with **non**secretory glands (Piearce, **1972;** Morgan, **1982).** Species belonging to the latter group appear to accumulate significantly higher Pb concentrations than members of the former species-group living in the same contaminated microenvironment (Morris and Morgan, **1985;** J. E. Morgan, in prep.). Competitive inhibition of Pb uptake by Ca has been offered as an explanation of the differences in Pb burdens of two pigmented species, *D. rubida* and *Lumbricus rubellus* (Ireland and Richards, **1977;** Morgan and Morris, **1982).** This conclusion has been reached by extrapolation from vertebrate studies (e.g. Baltrop and Khoo, **1976;** Hsu et *al.,* **1975).** Whether it is justified remains to be seen. Piearce **(1972)** did in fact detect a

FIGURE 1 (a and b) Diagrammatic representation of the hypothetical scheme that attempts to explain the mutual effects of Ca and Pb interactions in a soil-earthworm system, under calcareous (la) and acidic (lb) conditions. (The fact that the 'points' are connected does not necessarily imply that the respective compartments are directly connected. The slopes do not signify the magnitude of concentration **changes: they serve only to signify the likely direction of change.) See text for a description.**

higher assimilation of dietary Ca by L. *rubellus* (with active glands) compared with *Allolobophoru culiginosu* (inactive glands). Although it should be viewed circumspectly, it is nevertheless interesting that Zitko and Carson (1976) found that $Ca²⁺$ ions compete with heavy metal ions for active sites in fish.

Much the largest proportion of lead accumulates in the posterior alimentary region of earthworms (Ireland, **1975b).** Ultrastructural and X-ray analysis studies (Ireland and Richards, **1977;** Morgan and Morris, **1982)** have shown that the lead is accumulated, presumably **as** an insoluble product, in the calcium and phosphate-rich intracellular granules (chloragosomes) of the chloragogen tissue surrounding the intestine, major blood vessels and other internal organs.

Chloragosome granules possess cation-exchange properties (Fischer, **1973);** and it has been demonstrated both *in uifro* (Ireland, **1978)** and *in uiuo* (Morgan, **1984;** Morgan and Moms, **1982)** that Pb accumulation displaces Ca from the granules. This cationexchange may be analogous to the well-known displacement of Ca (and phosphate) from the bones of Pb-intoxicated vertebrates (Kato *ef ul.,* **1977).** Despite the loss of Ca **from** individual chloragosomes, an earthworm species with active calciferous glands *(L. terresfris)* is able to maintain its total-body Ca level within very narrow limits even when living in soils of widely different Ca content (Morris and Morgan, **1985)** i.e. they homeostatically equilibrate body Ca concentrations. Whether species with inactive glands can achieve this is not known.

B. Acidic soils (Figure 1 b)

Acidic soils in general probably support a restricted range of earthworm species (Piearce, **1972).** Acid spoil heaps associated with disused Pb/Zn mines tend to be nutrient deficient (Forstner and Wittmann, **1981),** and seldom therefore possess **a** deep soil layer. In our experience (Morgan and Morgan, in prep.) only the two litter-feeding pigmented species *D. rubidu* and *L. rubellus* are commonly encountered on such sites.

The solubilities of heavy metals may be considerably elevated under low pH conditions (Forstner and Wittmann, **1981).** Low pH and low soil Ca concentrations combine to increase the bioavailability of Pb, which probably explains the concentration factors higher than unity recorded by Ireland (197%) in *D. rubida* living in heavily polluted acidic soil.

In heavily polluted acidic soils it is proposed that the exceptionally high accumulated Pb concentrations within the worms may exceed the binding capacity of the chlorogosome compartment. The saturation of the chloragosomes, thus results in a 'spillage' of Pb into the chloragocytic cytoplasm, and perhaps ultimately to other tissues and organs. It should be emphasized that this is simply a hypothetical suggestion; experimental confirmation is not yet available. However, the appearance of Pb in the debris vesicles of chloragocytes in *D. rubida* from acid Pb-mine soil (Ireland and Richards, 1977) may reflect Pb spillage, since no Pb was detectable in the 'cadmosomes' (which are presumably homologous structures) of *D. rubida* from calcareous Pb-mine soil (Morgan and Morris, 1982).

Apart from the higher Pb concentration factors, the positive correlation between whole-body Ca and Pb concentrations (Ireland, 197%) distingusihes earthworms living in acidic Pb-polluted soils from populations of the same species living in calcareous Pbpolluted soils (Morgan and Morris, 1982). The increased wholebody Ca concentrations in earthworms living under acidic conditions must be explained in the light of a major cation-exchange displacement of Ca from individual chloragosome granules (Ireland, 1978; Morgan, 1984; Morgan and Morris, 1982). A likely consequence of Pb seepage from the saturated chloragosomes, if it occurs, is a generalized cell-membrane damage accompanied by the inhibition of the activities of various ATPases (see Tucker and Matte, 1980). The outcome of this Pb-induced cellular damage would be a depression of transcellular ion gradients, including the accumulation of intracellular Ca. Such a scenario has been proposed by Wrogemann and Pena (1976) and Trump *er* al. (1981) to explain the elevation of cellular, and especially intramitochondrial, Ca concentrations in experimentally- and pathologically-injured cells, including cells exposed to heavy metals (Trump et *af.,* 1981). In earthworms it is conceivable that these toxic effects may be exacerbated by the direct effect of Pb ions on the Ca regulatory functions of the calciferous glands, resulting in a general loss of Ca equilibration.

CONCLUSIONS

The very simple hypothetical scheme presented in this paper probably accounts for some of the known facts concerning Ca-Pb interactions involving earthworms and the soil substrate in which they live. However, this scheme most surely possesses severe limitations, perhaps inaccuracies, due to the paucity of definitive experimental data. An important justification of this presentation is that it brings into focus some of those areas where our knowledge is least reliable and complete. In particular it is important to know:

(i) *Geochemical considerations.* Do other inter-metal antagonisms affect Pb bioavailability and accumulation by earthworms, especially under acidic conditions? Cu and Fe may be implicated, for example (see Ireland, 1983).

(ii) *Trophic considerations.* What precisely do the different earthworm species eat (Piearce, **1978)?** Can the known species differences in whole worm metal burdens be explained by differences in: the metal **composition/availability** in their food materials; in gut passage-rate; assimilation efficiencies?

(iii) *The chlorugosomes.* Can the volume of this compartment expand in response to Pb accumulation? For example, this could effectively be achieved by: increasing the number of granules per cell; increasing the total cellular population; or by increasing cellular turnover rate.

(iv) *Excretory pathways.* Nothing is definitely known about the pathways, mechanisms, and relative rates of metal excretion in different earthworm species.

Answers to these and other questions should be obtained before worms can be used for pollution biomonitoring in terrestrial ecosystems.

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