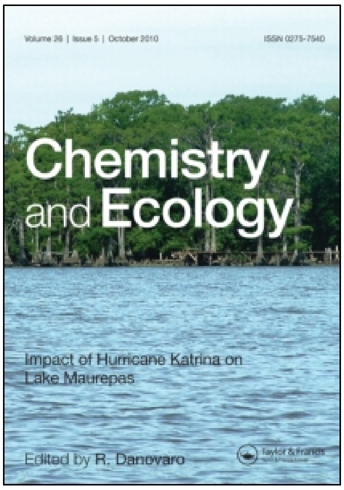

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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To cite this Article Morgan, A. J. , Morris, B. , James, N. , Morgan, J. E. and Leyshon, K.(1986) 'Heavy Metals in Terrestrial Macroinvertebrates: Species Differences Within and Between Trophic Levels', Chemistry and Ecology, 2: 4, 319 — 334
To link to this Article: DOI: 10.1080/02757548608080737
URL: http://dx.doi.org/10.1080/02757548608080737

Chemistry in Ecology, 1986, Vol. 2, pp. 319–334
0275-7540/86/0204-0319 $18.50/0

Printed in the United Kingdom

Heavy Metals in Terrestrial Macroinvertebrates: Species Differences Within and Between Trophic Levels

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(Received December 19, 1985; in final form March 6, 1986)

Whole animal concentrations of Pb, Zn and Cd were measured in herbivorous (1 snail species; 1 dipteran larva species), herbivorous + detritivorous (2 slug species), detritivorous (3 woodlouse species; 3 earthworm species), and carnivorous (1 carabid beetle species; 1 lithobiid centipede species) terrestrial macroinvertebrates collected at a disused Pb/Zn mine site. No evidence was found for an accretion of any metal during transference from herbivores to carnivores; the highest metal concentrations were, in fact, generally found in detritivores. The lack of metal bio-amplification during food chain transference is probably due to the sequestration of metals (notably Pb and Zn) in insoluble inorganic-rich granules within certain target organs and cells.

Earthworms and woodlice, respectively, showed major differences in metal concentrations. These differences between closely related species frequently exceeded differences between unrelated species occupying different trophic levels, and may be attributable to a combination of ill-defined ecological and physiological differences that ensure habitat and resource partitioning.

KEY WORDS Metals; macroinvertebrates; food-chains; pollution; biomonitoring:

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INTRODUCTION

An understanding of the ecological significance of organic and inorganic pollutants depends, not only on a knowledge of their concentrations in abiotic components of a habitat and in individual resident organisms, but also on a knowledge of their relative mobilities and bioaccumulation potentials within, and between, various trophic levels. To date, most transference studies have been performed in aquatic ecosystems (e.g. Bryan, 1979; Prosi, 1981). These reports demonstrate that the concentrations of organochlorine compounds, for example, tend to increase along the entire length of food chains. In contrast, the pattern for heavy metals, although to some extent metal-dependent, seldom displays true bioamplification at the carnivore trophic level.

Studies on aquatic organisms are complicated by the fact that they are exposed to environmental pollutants both in their diets and directly from their immediate surroundings (Moriarty, 1983). Terrestrial animals, however, are exposed almost exclusively to pollutants contained in their food.

A fairly limited number of studies have examined the flux of specific metals between producer-herbivore-detritivore-carnivore trophic levels in polluted terrestrial habitats (Williamson and Evans, 1972; Goldsmith and Scanlon, 1976; Martin and Coughtrey, 1976; Roberts and Johnson, 1978; Hunter and Johnson, 1982; Andrews and Cooke, 1984; Hunter *et al.*, 1984; Beyer *et al.*, 1985). The results of these published studies are not always consistent. Some reports indicate that there is no increase in the lead (Williamson and Evans, 1972; Roberts and Johnson, 1978; Beyer *et al.*, 1985) or cadmium (Andrews and Cooke, 1984; Beyer *et al.*, 1985) concentrations during transference to the carnivore trophic level. However, Roberts and Johnson (1978) suggested that cadmium concentrations are significantly higher in carnivores compared to herbivorous invertebrates. In this paper results are presented on the distribution of lead, zinc, and cadmium in selected macroinvertebrate species living in a heavily contaminated environment. The main objective was to attempt to resolve the conflict in the published literature on the flow of different heavy metals through terrestrial food chains, and to compare the results with published studies on the flow of metals in aquatic communities, to see whether general principles can be established.

Our understanding of metal transference along terrestrial food chains is restricted for at least three reasons. First, an inadequate knowledge of the precise food selection (Hunter and Johnson, 1982) and assimilatory characteristics of individual species (Hopkin *et al.*, 1985). Second, the concepts of 'food chains' and 'trophic levels' are restrictive and misleading, because they imply that an organism consumes materials from its adjacent lower trophic level; in the case of carnivores they can of course obtain food from several different lower trophic levels. Third, in most investigations the collected organisms have usually been pooled into broad trophic and taxonomic groups for the purposes of analyses and modelling, with scant regard to the significant differences that may exist in the metal concentrations of related species. However, recent observations on woodlice (Hopkin *et al.*, 1985) and earthworms (Beyer *et al.*, 1985; Morris and Morgan, 1985) show that inter-species differences in metal concentrations can be large, and may exceed the differences between unrelated species occupying distinct trophic levels.

A second objective of this paper was to measure and compare differences in the lead, zinc and cadmium concentrations of different slug, woodlouse, and earthworm species, respectively. We also discuss the implications of these differences between closely related species to metal transference studies, and the validity of using invertebrates for pollution biomonitoring purposes.

METHODS

Selected macroinvertebrates were collected by hand from a small mixed-deciduous woodland, near the centre of a disused Pb/Zn mine-site at Llantrisant, South Wales (British Ordnance Survey Reference Number ST 048 823).

Earthworms were transferred to the laboratory in their native substrates; washed briefly in tap water to remove adherent soil; and their gut contents cleared by maintenance on moistened filterpaper (deionized H_2O; Whatman No. 1) in a dark constant-temperature (10°C) cold room for 4 days. The filter paper was changed at least daily. All other invertebrates were briefly rinsed in deionized water to remove all external adherent soil and litter particles. The animals, including earthworms, were killed by immersion in liquid N_2, and stored in a deep freeze cabinet prior to analysis. The

TABLE I
A list of the macroinvertebrates collected and analyzed from the Llantrisant mine site, with a summary of their supposed diets*

Species	Code		Taxonomic group	Diet	Trophic group
Hygromia hispida	Hh	(P)	Mollusca	Seedlings, young shoots[1]	HERBIVORES
Tipula paludosa (larva)	Tp	(C) (O)	Insecta, Diptera	plant roots[1]	
Deroceras caruanae	Dc	(P) (C)	Mollusca Gastropoda	Mainly fresh plant material; but also some (dead) animal materials[2]	HERBIVORES + DETRITIVORES
Deroceras reticulatum	Dr	(S-C) (O)	Pulmonata Stylommatophora		
Polydesmus angustus	Pa	(C)	Diplopoda	A primary decomposer; may also consume some living plant tissues[3]	DETRITIVORES
Porcellio scaber	Ps	(C)	Crustacea	Primary and secondary decomposers[4]	
Philoscia muscorum	Pm	(O)	Isopoda		
Oniscus asellus	Oa				
Dendrobaena mammalis	Dm	(P)	Annelida	Primary decomposers of plant, root, stem and leaf materials; also consume seeds, algae, fungi, Protozoa and microbes[5]	
Lumbricus rubellus	Lr	(C)	Oligochaeta		
Allolobophora caliginosa	Ac	(F)	Lumbricidae		
Pterostichus madidus	Pt	(C) (O) (F)	Insecta Coleoptera Carabidae	(Primarily carnivorous; broad spectrum of prey species)	CARNIVORES
Lithobius variegatus	Lv	(C) (O)	Chilopoda Lithobiomorpha	A range of small invertebrates, including mites, oplionids, Collembola, enchytraeids, woodlice. May possibly also consume some litter, especially in Winter[6]	

* The (often) imprecise information on the diets of individual species has been derived from the following published sources: 1 Cloudsley Thompson & Sankey (1968); 2 Pallant (1968); 3 Edwards (1974); 4 Sutton (1972); 5 Piearce (1978); 6 Lewis (1981).

invertebrates examined in this study and their suggested diets are listed in Table I.

Soils (0–5 cm depth samples), litter, and animals were processed for atomic absorption spectrophotometry (Pye/Unicam SP 2900; Varian-Techtron AA6) by wet-digestion in boiling, concentrated 'Analar' grade nitric acid (BDH Chemicals, Poole, Dorset, UK). A hydrogen lamp was used to automatically compensate for non-atomic absorption: the methodology has been described in more detail elsewhere (Morgan and Morris, 1982).

RESULTS AND DISCUSSION

The soil and litter samples collected at Llantrisant were heavily contaminated with lead and zinc, whilst the cadmium concentrations were much lower but still higher than expected background levels (Table II).

The metal concentrations in individual species are presented in Table III, and graphically compared in Figure 1(a–c). Concentration factors (expressed as the ratio of the total animal metal concentration: soil metal concentration) for cadmium were well above unity in most instances. Lead concentration factors, however, were invariably lower than unity. The high cumulative potential of cadmium within terrestrial animal communities is an established phenomenon (Martin and Coughtrey, 1976; Roberts and Johnson, 1978; Williamson, 1979; Hunter and Johnson, 1982).

No evidence was obtained in the present study for a bioamplification of lead, zinc or cadmium during transference to carnivores (Figure 1(a–c)), although the lead and zinc concentrations tended to be higher in the detritivores compared with herbivores (Fig. 1a, c). These findings are in accord with the observations of Prosi (1981) on metal distribution in a freshwater community, and with independent published observations on the distribution of lead (Williamson and Evans, 1972; Roberts and Johnson, 1978) and zinc (Roberts and Johnson, 1978) in terrestrial communities.

The bioavailability of certain non-essential heavy metals, such as lead, becomes diminished during passage from organisms occupying low to organisms occupying the highest trophic level. A likely reason for this is the presence of specific and highly efficient metal-binding ligands in the cells and tissues of all invertebrate phyla (Morgan, 1984), which render the bound metals less available to predatory animals. These ligands are frequently located within discrete, but structurally and compositionally diverse, intracellular

TABLE II
Concentrations of heavy metals ($\mu g\,g^{-1}$ dry weight, Mean ± Standard Error) in soil and litter from the Llantrisant mine microhabitat

	Pb	Zn	Cd	Ca
Litter	465 ± 89 (6)	427 ± 91 (6)	7.3 ± 1.5 (6)	3380 ± 340 (5)
Soil	8740 ± 833 (6)	2210 ± 96 (6)	16.4 ± 0.4 (6)	6630 ± 498 (6)

TABLE III

Concentrations of heavy metals ($\mu g\,g^{-1}$ dry weight, Mean ± Standard Error) i macroinvertebrate species from the Llantrisant mine site

		Pb	Zn	Cd	Ca
Hygromia hispida	(SNAIL)	176 ± 15 (10)	437 ± 61 (5)	25 ± 3 (10)	240430 ± 18240 (5)
Tipula paludosa	(DIPTERAN larva)	439 ± 104 (5)	483 ± 68 (5)	33 ± 3 (5)	—
Deroceras caruanae	(SLUG)	363 ± 22 (10)	515 ± 36 (10)	53 ± 10 (10)	12070 ± 316 (10)
Deroceras reticulatum	(SLUG)	254 ± 16 (10)	619 ± 22 (10)	37 ± 3 (10)	13270 ± 219 (10)
Polydesmus angustus	(MILLIPEDE)	47 ± 41 (5)	406 ± 228 (5)	–	69010 ± 11975 (5)
Porcellio scaber	(WOODLOUSE)	22 ± 11 (10)	1005 ± 893 (10)	22 ± 7 (10)	149090 ± 27520 (10)
Philoscia muscorum	(WOODLOUSE)	543 ± 255 (11)	130 ± 87 (11)	57 ± 20 (11)	133270 ± 47620 (11)
Oniscus asellus	(WOODLOUSE)	813 ± 192 (10)	299 ± 122 (10)	72 ± 21 (10)	249950 ± 36860 (10)
Dendrobaena mammalis	(EARTHWORM)	502 ± 100 (10)	621 ± 49 (10)	60 ± 7 (10)	3683 ± 253 (10)
Lumbricus rubellus	(EARTHWORM)	696 ± 107 (10)	1187 ± 180 (10)	66 ± 6 (10)	4902 ± 309 (10)
Allolobophora caliginosa	(EARTHWORM)	5335 ± 571 (10)	1280 ± 136 (10)	157 ± 22 (10)	2293 ± 229 (10)
Pterostichus madidus	(BEETLE)	62 ± 7 (10)	248 ± 36 (6)	5 ± 0.7 (10)	1190 ± 53 (6)
Lithobius variegatus	(CENTIPEDE)	480 ± 79 (6)	1608 ± 292 (6)	52 ± 12 (6)	2580 ± 289 (6)

Numbers in parentheses = number of observations; — = not analyzed.

granules or spherites (Morgan, 1984). Some of these serve as storage pools for essential metals such as zinc and copper; others represent effective detoxification depositories for toxic metals such as lead. A functionally important property of the granules that are able to bind heavy metals, is that they are insoluble under aqueous conditions. For example, intracellular granules in the hepatopancreas of terrestrial snails possess pyrophosphate groups, which are not only able to bind several different metals, including lead and zinc, but form extremely insoluble complexes with them (Simkiss, 1981a, 1981b). Thus, it is very significant that a major proportion of the lead and copper content of woodlice cannot be assimilated by centipedes (Hopkin and Martin, 1984a) and spiders (Hopkin and Martin, 1985) feeding on them. This is due to the immobilization of the metals by sulphur-rich ligands within the woodlouse hepatopancreas (Hopkin and Martin, 1982a, 1984b).

There is a growing awareness, based on compelling supportive evidence (e.g. Ma, 1982; Morgan, 1985), that the availability of heavy metals to the biota of an ecosystem is determined by the absolute concentration of the metal in the abiotic compartment(s), and also by the chemical form or 'species' of the metal. We suggest that this biogeochemical principle can be extrapolated to account for some of the metal fluxes between organisms occupying different trophic levels. The amount of metal assimilated by an animal is,

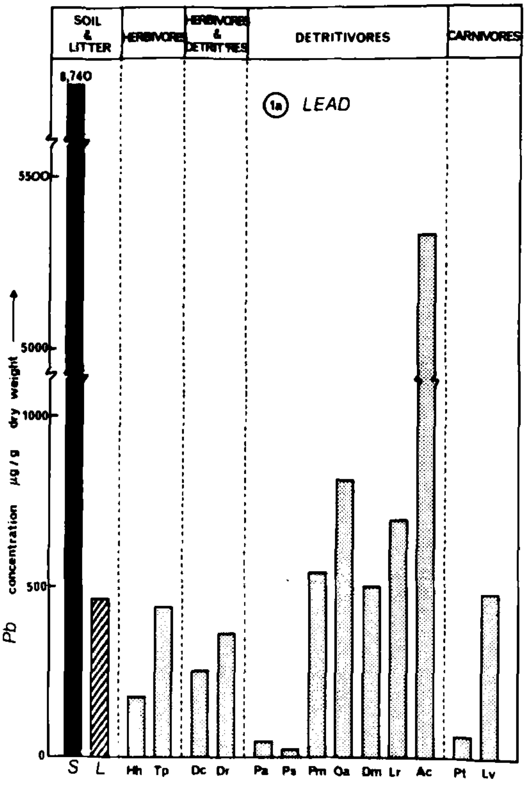

FIGURE 1 Mean concentrations of (a) lead, (b) zinc, and (c) cadmium in soil (S), litter (L) and selected macroinvertebrates. Hh = *Hygromia hispida*, Tp = *Tipula paludosa*, Dc = *Deroceras caruanae*, Dr = *D. reticulatum*, P = *Polydesmus angustus*, Ps = *Porcellio scaber*, Pm = *Philoscia muscorum*, Os = *Oniscus asellus*, Dm = *Dendrobaena mammalis*, Lr = *Lumbricus rubellus*, Ac = *Allolobophora caliginosa*, Pt = *Pterostichus madidus*, Lv = *Lithobius variegatus*. Refer to Table III for S.E. and n values; results of statistical analyses are summarized in Table IV.

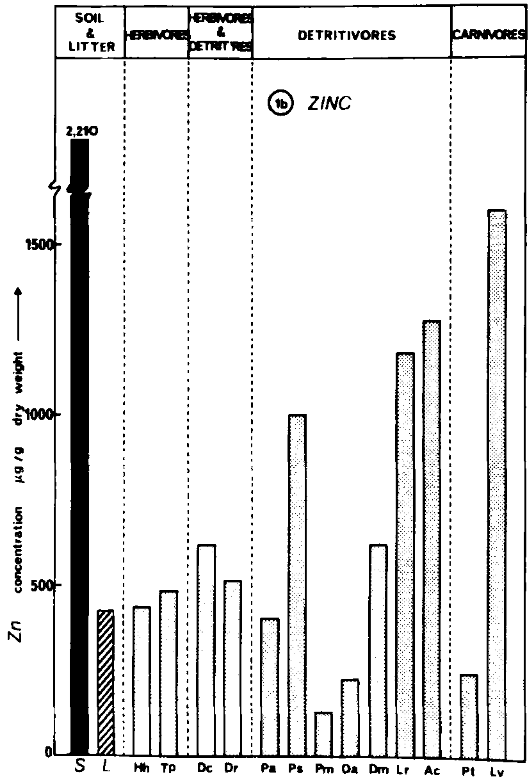

FIGURE 1b

therefore, largely a reflection of the bioavailability of the metal in the food material, rather than a direct function of the dietary metal concentration (see Hopkin and Martin, 1984a).

Although Roberts and Johnson (1978) appear to agree with the present observations (Figure 1(a)) that there is no overall amplification of lead within terrestrial food chains, they did report higher lead concentrations in carnivorous compared with herbivorous invertebrates. They attributed this enhancement to the deposition of lead in calcium-rich skeletal components of the carnivores. Lead does indeed accumulate in the skeletons of vertebrates (Kato *et al.*, 1977), but there is little evidence that it accumulates in the calcium carbonate impregnated exoskeletons of many invertebrates. Simkiss

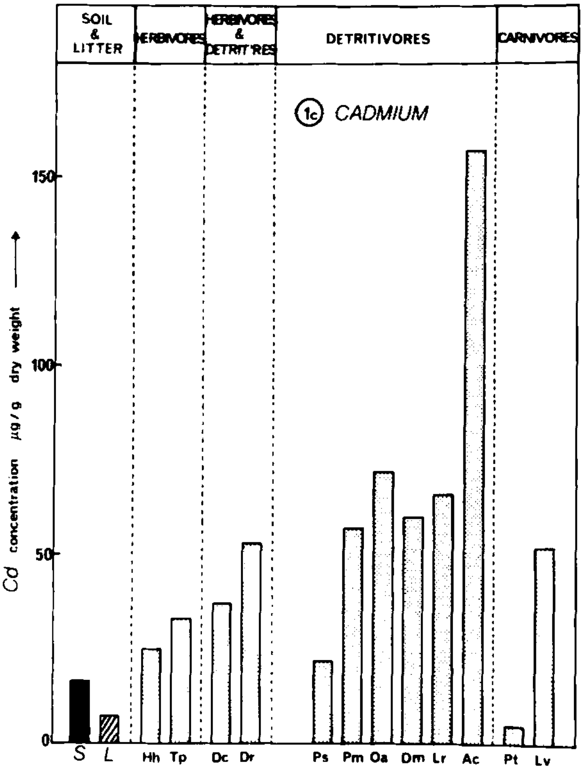

FIGURE 1c

(1976; 1981a) has indicated that in general heavy metals have a stronger affinity for phosphate-rich rather than carbonate-rich minerals. Significantly, therefore, there appears to be no correlation between the presence of a heavily calcified exoskeleton and the amount of lead accumulated by the whole animal (Table III). Furthermore, it has been shown that only minor proportions of the body burdens of lead, and other heavy metals, are deposited in the exoskeletons of woodlice (Hopkin and Martin, 1982b) and terrestrial snails (Avery *et al.*, 1983).

Since the exoskeleton frequently accounts for a very large proportion of the dry body weight of some invertebrates, and yet it evidently does not represent a significant heavy metal containing compartment, should it be included in metal analyses? Both Prosi

(1981) and Moriarty (1983) argue that all comparisons of metal transfer through ecosystems must be based on compatible data derived from each trophic level. This implies that whole animals, including their calcified exoskeletons, should be analyzed because it is clearly impossible to obtain equivalent tissue and organ samples from diverse animal groups. The ecological argument favouring the analysis of whole animals is based on the assumption that predators consume whole prey and not selected portions of them. When this is the case, a predator consuming calcareous prey will be simultaneously exposed to sequestered heavy metals in the soft tissues, and to relatively soluble calcium salts derived from the exoskeleton. Of course, some predators such as spiders do not consume their entire prey (Hopkin and Martin, 1985). Nevertheless, since metal interactions (for example, between Ca–Pb, and Cu–Pb) certainly affect metal uptake by detritivorous invertebrates (Beeby, 1978; Ireland, 1983), the existence of analogous interactions may also affect the amount of specific metals assimilated by carnivores whose food often contains highly concentrated (albeit 'immobilized') mixtures of several metals.

Whole animals were analyzed in the present study. The inclusion of their heavily calcified exoskeletons, results in a significant underestimate of soft-tissue metal concentrations in the herbivorous snail *H. hispida*, the millipede *P. angustus*, and in the three woodlouse species compared to the concentrations in both carnivores (Table III). This consideration lends further support to the conclusions drawn from Figure 1(a–d) that the concentrations of lead, zinc, and cadmium do not increase progressively throughout their trophic transference pathways.

The pattern of metal transference may conceivably be metal dependent. Cadmium, unlike lead, is not accumulated by animals as an insoluble and predominantly inorganic product. It is usually sequestered intracellularly by inducible low-molecular weight proteins (Roesijadi, 1981). Organically-bound metals, including cadmium and mercury, may tend to accumulate within higher trophic constituents, rather like fat-soluble organic pesticides such as DDT (Prosi, 1981), because metalloproteins may be hydrolysed by digestive enzymes in the carnivore gut. This supposed enhanced availability, coupled with a low excretory rate, may account for the higher cadmium concentrations recorded in carnivores compared to herbivores by Roberts and Johnson (1978). However, our cadmium observations (Table III; Figure 1(c)) fail to substantiate Roberts and Johnson's (1978) findings. We did find that cadmium concentrations were generally higher in detritivores compared to herbivores (Figure 1(c)), an observation similar to that made by Prosi (1981) for a river community. Further work, of a much more detailed and systematic nature, is necessary to resolve the issue of possible metal differences in transfer patterns. These studies must examine and define the food selection and consumption habits of carnivores, in particular. For example, if carnivores derive most of their prey from the herbivore trophic level, and especially if they consume juveniles (see Lewis, 1981), the resultant analyses could well reveal a true net amplification of metal concentrations within the established food chain.

The present study observed some very significant differences between the metal concentrations of unrelated (Figure 1(a–c)) and closely related species (Table IV; Figure 2) of detritivores, respectively. Millipedes assimilated much less lead and cadmium than two

TABLE IV

Statistical analysis (2-tailed t-tests) of the differences between mean metal concentrations* in related species of detritivorous macroinvertebrates from Llantrisant

		Pb	Zn	Cd	Ca
SLUGS	*Deroceras reticulatum** *Deroceras carunae*	$P<0.001$	$P<0.05$	N.S.	$P<0.01$
WOODLICE	*Oniscus asellus* *Porcellio scaber*	$P<0.001$	$P<0.05$	$P<0.001$	$P<0.001$
	Oniscus asellus *Philoscia muscorum*	$P<0.05$	$P<0.05$	N.S.	$P<0.001$
	Porcellio scaber *Philoscia muscorum*	$P<0.001$	$P<0.01$	$P<0.001$	N.S.
EARTHWORMS	*Lumbricus rubellus* *Dendrobaena mammalis*	N.S.	$P<0.01$	N.S.	N.S.
	Lumbricus rubellus *Allolobophora caliginosa*	$P<0.001$	N.S.	$P<0.001$	$P<0.001$
	Dendrobaena mammalis *Allolobophora caliginosa*	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$

*See Table 3; N.S. = Not significant at the 5% level of significance; * = these slugs also consume fresh vegetation.

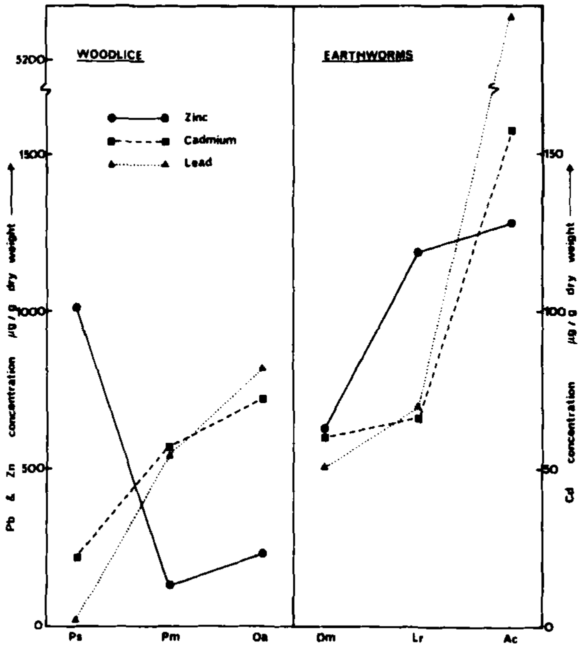

FIGURE 2 Species differences in the lead, zinc, and cadmium concentrations of three species of woodlice and three species of lumbricid earthworms, respectively. (Code letters as in Table I and Figure 1).

of the woodlouse species and all three earthworm species. In comparison, the differences in zinc concentrations both within and between trophic levels was not as marked as for the toxic metals (Figure 1(a–c)), suggesting that the invertebrates may be able to regulate their zinc burdens. The extremely high zinc concentration in the centipede is worthy of comment, especially since this carnivore did not appear to assimulate much lead or cadmium. Other workers (Hopkin and Martin; Beyer *et al.*, 1985) have also recorded high zinc concentrations in centipedes, where it is associated with the fat body and exoskeletal elements (Hopkin and Martin, 1983).

Striking species differences were observed in the concentrations of lead, zinc, and cadmium in woodlice and earthworms (Table IV; Figure 2). Hopkin *et al.* (1985) also found qualitatively similar concentration differences in three isopod species, including *O.*

asellus and *P. scaber*. They suggested that since these closely related species probably eat similar food items, the observed differences in metal assimilations are due to certain (unknown) differences in digestive physiology. Hopkin *et al.* (1985) speculated that, for example, relatively minor differences in gut pH would result in large differences in the solubility, and hence potential availability, of lead. Whatever the cause of these differences, it is interesting to note (Figure 2) that the three heavy metals are disproportionately assimilated. In *P. scaber* zinc is highly assimilated but lead and cadmium are both low; *O. asellus* assimilates relatively high quantities of lead and cadmium, but little zinc; *P. muscorum* is approximately intermediate. The possibility that the three species are somehow partitioning their food resources cannot be dismissed.

Differences in burrowing activities, food selection, calcium metabolism and digestive physiology (Piearce, 1972; Bengtsson *et al.*, 1983; Morris and Morgan, 1985) probably all contribute to the species differences in metal concentrations observed in the three earthworm species (Table IV; Figure 2). For example, the non-pigmented soil/humus consuming species *A. calignosa* has significantly higher lead and cadmium concentrations than the ecophysiologically distinct litter consumers, *D. mammalis* and *L. rubellus*. Perel (1977) demonstrated thát soil/humus consumers have a structurally more elaborate typhlosole than the litter consumers. This increase in the absorptive surface-area of the gut, together with the higher rate of throughput of gut contents in *A. calignosa* compared to *L. rubellus* (Piearce, 1972), may result in a higher assimulation of nutrients and metals by species such as *A. calignosa*.

Two general conclusions may be drawn from the results of this study. First, it reaffirms the conclusion of Hopkin *et al.* (1985) that if invertebrates such as woodlice (Martin and Coughtrey, 1982b) and earthworms (Helmke *et al.*, 1979; Kruse and Barrett, 1985) are to be used for monitoring environmental metal pollution, then it is essential that the same species is compared consistently at different sites. Second, it highlights the need for better differentiated studies that identify the precise quality and quantity of food consumed by various organisms in their own microhabitat. This information, allied to laboratory metal-assimilation studies, will improve our present rather poor understanding of the processes regulating the flux of heavy metals within food webs.

Acknowledgements

We thank Sabina Thompson for typing the manuscript, and Mr Viv Williams for photographic assistance. This work was performed during the award of a Research Studentship by the Natural Environment Research Council to J. E. Morgan.

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