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THE ASSIMILATION OF HEAVY METALS BY *LITHOBIUS VARIEGATUS* AND *GLOMERIS MARGINATA* (CHILOPODA; DIPLOPODA)

by

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ABSTRACT

Specimens of the centipede *Lithobius variegatus* and the millipede *Glomeris marginata* collected from uncontaminated sites, and sites contaminated with heavy metals, have been fed on tissues from the terrestrial isopod *Oniscus asellus* and leaf litter, respectively, containing different concentrations of zinc, cadmium, lead and copper. The extent to which these elements are accumulated by *Lithobius variegatus* or *Glomeris marginata* depends on the amounts of metals in the food and the degree of contamination of the site from which the centipedes and millipedes are collected. The tissues within the body where metals are stored are compared in the two animals and suggestions are made of the areas which are in most need of further research.

INTRODUCTION

One of the world's largest primary zinc, lead and cadmium smelting works is situated at Avonmouth, 10 km to the north-west of Bristol in south-west England. Despite the presence of sophisticated filtering equipment which removes in excess of 98% of the metals from the waste gases, large amounts of zinc, cadmium, lead and copper are emitted each year as fine particles, some of which are carried more than 20 km to the north-east by the prevailing south-westerly winds.

Our studies have been concerned mainly with the impact of this pollution on the ecology of two oak-hazel woodlands, Hallen Wood and Haw Wood, which are situated on either side of the M5 motorway, 3 km downwind of the smelting works. These woodlands are heavily contaminated with zinc, cadmium, lead and copper which reach concentrations in the leaf litter which are 10 to 20 times greater than in similar uncontaminated woodlands. Concurrent with this contamination is a thick layer of undecomposed leaf litter which represents more than 20 years of accumulation (Coughtrey et al., 1979).

A study of the soil/litter fauna has shown clearly that there are differences in the extent to which different taxonomic groups of invertebrates are affected by the pollution. Some groups such as millipedes and earthworms are reduced greatly in numbers whereas others such as centipedes and large woodlice are apparently unaffected (table I). The reduction in the numbers of millipedes and earthworms almost certainly accounts for the reduction in decomposition of litter at the contaminated sites since previous studies have shown that there is no reduction in abundance or diversity of microorganisms and fungi as a result of the pollution (Martin et al., 1980). The springtails, mites, Diptera larvae and predatory beetles are present in greater numbers per unit area, probably because the larger standing crop of leaf litter supports a larger amount of fungal material on which mites and springtails feed, which in turn provide more prey for predatory insects.

In this presentation, experiments are described on the assimilation of heavy metals by the centipede *Lithobius variegatus* Leach which is common in the contaminated woodlands, and the pill millipede *Glomeris marginata* (Villers) which is absent (table I). The detailed results of this work will be presented elsewhere (Hopkin & Martin, 1984a; Hopkin et al., in prep.). However, the purpose of this paper is to summarise the main findings of this work, to compare for the first time the assimilation of zinc, cadmium, lead and copper in *Lithobius variegatus*

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TABLE I

Density of major invertebrate groups (number of individuals m^{-2}) in leaf litter and soil from a contaminated woodland 3 km downwind of a smelting works (Hallen) and a similar but uncontaminated site (Wetmoor).

		Hallen	Wetmoor
Litter standing crop (kg m^{-2} dry wt.)		14.28	1.35
Isopoda	*Oniscus asellus* Linnaeus	56	20
	Trichoniscus pusillus Brandt	0	151
Diplopoda	Polydesmidae	8	79
	Julidae	0	11
	Glomeridae	0	21
Chilopoda	Lithobiidae	112	116
	Geophilomorpha	328	263
Arachnida	Acari	129000	19400
	Araneae	248	81
	Pseudoscorpionidae	200	67
Insecta	Collembola	20800	8688
	Coleoptera	902	48
	Coleoptera (larvae)	120	4
	Diptera (larvae)	4590	291
Annelida	*Lumbricus rubellus* Hoffmeister	17	3
	Lumbricus terrestris Linnaeus	0	29
	Allolobophora longa Ude	0	4
	Allolobophora caliginosa Savigny	0	30
	Octolasium cyaneum Savigny	0	9

and *Glomeris marginata* and to suggest areas which are in most need of further research.

MATERIALS AND METHODS

Lithobius variegatus

Specimens of adult *Lithobius variegatus* and the woodlouse *Oniscus asellus* (Linnaeus) were collected from Wetmoor Wood (British Ordnance Survey grid reference ST 743 876) and Haw Wood (ST 560 798) during May 1982. Wetmoor is an uncontaminated site, whereas Haw is 3 km downwind of a primary smelting works and is heavily contaminated with zinc, cadmium, lead and copper (Martin et al., 1982).

The woodlice were maintained in plastic tanks on leaf litter from the collection site. The centipedes were starved for four days to allow the contents of the gut to be voided. At the end of this period, fourteen centipedes of 2-3 cm in length were selected from each site population and transferred to individual Petri dishes. The dishes were kept on a moist base within a covered plastic tank and were thus all maintained at the same relative humidity and temperature (14-18 °C) for the duration of the experiment.

The hepatopancreas of *Oniscus asellus* consists of four blind-ending tubules. Within individual animals, the concentrations of zinc, cadmium, lead and copper are the same in all four lobes (Hopkin & Martin, 1982b). Therefore, if the left pair are removed and fed to a centipede, the amounts of metals in the food can be determined from the concentrations in the right pair of tubules.

Within each of the two groups of fourteen centipedes, seven were fed on tubules from the hepatopancreas of woodlice from Wetmoor and seven on tubules from the hepatopancreas of woodlice from Haw. The tissue was presented to each animal on a small piece of woodlouse cuticle which does not contain detectable amounts of zinc, cadmium, lead or copper. The other two lobes were stored on a small piece of Millipore filter paper. Each centipede grasped the piece of cuticle with the poison claws and ate the soft tissue, usually within about ten minutes. The piece of cuticle, which was never eaten, was removed on the following day. During the next three days, the faeces voided by the centipedes were collected for analysis. The amounts of metals taken in by the centipedes could be determined accurately by subtracting the amounts of metals remaining on the piece of cuticle from the amounts in the opposite pair of hepatopancreas tubules. The cen-

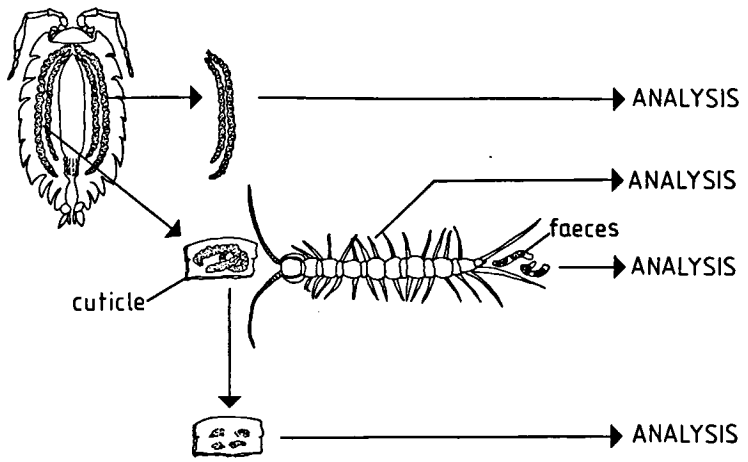


Fig. 1. Diagrammatic representation of the experimental procedure used to measure the assimilation of metals by *Lithobius variegatus* (for further details see text).

tipedes were fed on six occasions at intervals of four days. The experimental design is summarised in fig. 1.

Centipedes which died during the experiment were dissected immediately. However, after the sixth feeding occasion, all the centipedes from Wetmoor fed on woodlice from Haw had died (table II). Centipedes which were still alive at this stage were starved for four days and dissected also. The guts of all centipedes were empty when removed from the body cavity.

TABLE II

Number of specimens of *Lithobius variegatus* surviving after eating two lobes of the hepatopancreas of six specimens of *Oniscus asellus* (n = 7 at start of experiment).

		Lithobius variegatus	
		Haw (con-taminated)	Wetmoor (uncon-taminated)
Oniscus asellus	Haw	5	0
	Wetmoor	7	7

Each centipede was dissected into ten tissue fractions (Hopkin & Martin, 1983). However, for the sake of simplicity, the results of the analyses have been amalgamated into three fractions in this paper, 'gut', 'exo' (the exoskeleton including the connective tissues adhering to the inner surface) and the 'rest' fraction consisting of a pooled sample of all the other organs in the body cavity. In addition, seven centipedes were collected from each of the field sites within a few days of the end of the experiment, starved for four days and dissected in the same manner.

The tissues and samples of faeces were placed on small pieces of Millipore filter paper, dried overnight at 70°C and weighed on a microbalance. The samples were digested in 2 ml of boiling Aristar grade concentrated nitric acid and diluted to 5 ml with deionised distilled water. The digests were analysed for zinc, cadmium, lead and copper by flame (Varian AA775) or flameless (Varian GTA95) atomic absorption spectrophotometry (AAS).

Figures for the net fluxes of metals through *Lithobius variegatus* between the beginning and end of the experiment (figs. 2-4) were calculated by balancing the amounts measured in the faeces with changes in the levels in the tissues on the assumption that the concentrations prior to the first feeding occasion were the same as in centipedes collected from the field. The estimates of the amounts of metals in the food calculated on this basis and shown on the left hand side of the figures were in good agreement with the amounts of metals in the opposite pair of tubules of the hepatopancreas of *Oniscus asellus* fed to the centipedes.

Glomeris marginata

Specimens of *Glomeris marginata* were collected from Midger Wood (ST 796 893) and from Charterhouse (ST 503 555) on the Mendip Hills to the south of Bristol during October 1983. Midger Wood is an uncontaminated oak-hazel woodland, whereas Charterhouse is rough grassland on the spoil tips of a disused lead mine contaminated with zinc, cadmium, lead and copper (Hopkin & Martin, 1982a).

Groups of twelve millipedes from each population were placed in factorial combination in individual Petri dishes

on leaf litter from Midger Wood (field maple, *Acer campestre* L.), Charterhouse (grass) and Haw Wood (field maple) from which *Glomeris marginata* is absent. The millipedes were maintained for two months in plastic tanks in the same manner as *Lithobius variegatus*. Faecal pellets were removed every few days and stored for analysis.

At the end of the experiment, only a small number of the millipedes had died (table III). The survivors were dissected into three tissue fractions, 'gut' (from which the contents were removed), 'fat body' and a pooled 'rest' sample which consisted of the exoskeleton and other organs within the body cavity. In addition, twelve millipedes were collected from each of the field sites within a few days of the end of the experiment and dissected in the same manner. The concentrations of zinc, cadmium, lead and copper in the tissue fractions, and the fluxes between tissues during the experiment were calculated in the same way as for *Lithobius variegatus*. However, it proved difficult to determine the consumption of leaf litter by weight loss of leaves in the Petri dishes between the beginning and end of the experiment due to fluctuations in the wet weight of this material. Consequently, the relative rates of consumption of food were determined from the weight of faeces produced.

TABLE III

Survival and daily production of faeces as a percentage of body weight by *Glomeris marginata* from an uncontaminated woodland (Midger) and a disused lead mine (Charterhouse) fed on contaminated and uncontaminated leaf litter for two months (n = 12 at start of experiment).

Millipedes	Leaf litter	n surviving	faeces production (%)
Midger Wood	Midger Wood	10	6.4
	Charterhouse	11	6.4
	Haw Wood	12	3.0
Charterhouse	Midger Wood	12	10.4
	Charterhouse	11	10.0
	Haw Wood	12	2.0

RESULTS AND DISCUSSION

Lithobius variegatus

Centipedes from the contaminated site survive longer than those from the uncontaminated site when both populations are fed on the hepatopancreas of *Oniscus asellus* containing high concentrations of metals (table II). This suggests that *Lithobius variegatus* from the contaminated site have acquired tolerance to elevated levels of metals in their diet.

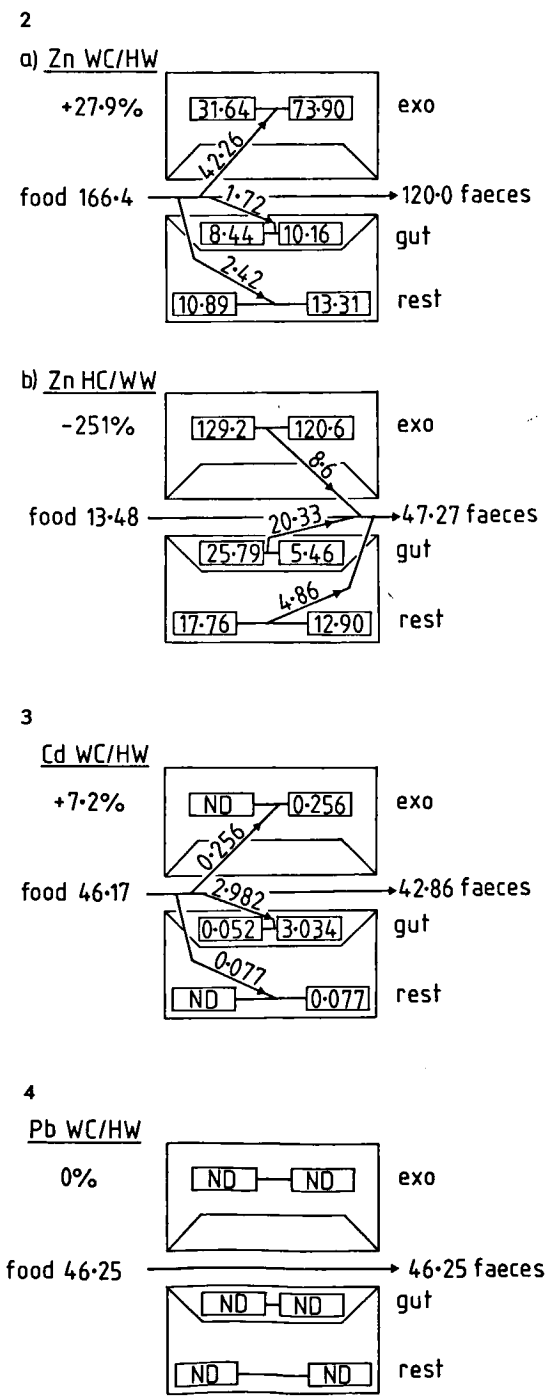
The extent to which some metals are assimilated depends on both the levels in the food and the degree of contamination of the site from which the centipedes were collected. For example, if specimens of *Lithobius variegatus* eat food which contains higher levels of zinc than they would normally expect in their diet, they assimilate the metal (fig. 2a). However, if they consume food containing lower levels, they lose three times more zinc in the faeces than they take in with the hepatopancreas tubules (fig. 2b). Most of this metal is lost from the gut and it is interesting to note that our preliminary observations on the ultrastructure of this organ have revealed the presence of intracellular granules in which calcium, phosphorus and zinc can be detected by X-ray microanalysis. These granules can also be found in the faeces and may provide a route by which excess zinc can be removed from the body.

There are important differences in the extent to which different organs take up metals which would be overlooked if only overall assimilation rates were considered. For example, *Lithobius variegatus* from Wetmoor assimilate only 7.2% of the cadmium when fed on the hepatopancreas of woodlice from Haw (fig. 3). However, the concentration of this metal in the gut increases by more than 50 times during the experiment. The epithelial cells of this organ are clearly acting as a barrier to the passage of this potentially toxic element into the rest of the body tissues.

Lead is not assimilated by *Lithobius variegatus*, even when the centipedes are fed on the tissues of woodlice containing much higher concentrations of this element than they would expect in the wild (fig. 4). However, it is not known whether this is due to the lead being in an insoluble form in the digestive fluids of the centipedes, or whether they possess specific mechanisms which prevent its uptake.

The concentrations of cadmium and copper increase in the gut tissues of centipedes fed on the hepatopancreas of woodlice from their

'own' site. This suggests that adult *Oniscus asellus* do not form a major proportion of the diet of *Lithobius variegatus* in deciduous woodlands although they probably consume large numbers of newly-released juvenile woodlice in which the concentrations of metals are much lower (Hopkin & Martin, 1984b).



Figs. 2-4. Estimated assimilation rates (%) and net fluxes in amounts of metals through *Lithobius variegatus* and between the 'gut', 'exo' and 'rest' tissue fractions between the beginning and end of the experiment. The value given in the left hand box of each tissue fraction represents the amount of metal estimated to be present at the start of the experiment. The value in the right hand box represents the amount of metal present at the end. (ND, not detected.) 2a: Zinc (nmol) in Wetmoor centipedes fed on Haw woodlice (WC/HW). 2b: Zinc (nmol) in Haw centipedes fed on Wetmoor woodlice (HC/WW). 3: Cadmium (nmol) in Wetmoor centipedes fed on Haw woodlice (WC/HW). 4: Lead (nmol) in Wetmoor centipedes fed on Haw woodlice (WC/HW).

Glomeris marginata

Glomeris marginata from both sites ate significantly less leaf litter from Haw Wood than from Charterhouse or Midger Wood (table III). Presumably, they find some aspect of the composition of the leaves, which might include the high concentrations of metals, 'distasteful'. Interestingly, *Glomeris marginata* from Charterhouse ate more of both types of leaf litter than millipedes from Midger although the reason for this difference is not clear. All the millipedes initially lost weight in the first two weeks of the experiment although those fed on litter from Midger and Charterhouse showed signs of recovery of this weight loss towards the end of the experiment. *Glomeris marginata* fed on leaf litter from Haw did not recover this initial weight loss, probably due to their low relative consumption of food.

The 'rest' fraction was by far the most important tissue fraction for the storage of zinc (fig. 5) and copper, whereas for cadmium (fig. 6), significant amounts of this metal were also contained in the gut. It is clear that the gut epithelium of *Glomeris marginata* is far less successful at preventing the passage of cadmium from the food in the lumen across the epithelium into the body tissues than the gut of *Lithobius variegatus*. The fat body of *Glomeris marginata* is not of major importance in the storage of any of the four metals.

Glomeris marginata, like *Lithobius variegatus*, does not assimilate significant amounts of lead, even when feeding on leaf litter which contains concentrations of this metal many times greater than they would experience in the wild (fig. 7).

Glomeris marginata from Charterhouse consistently assimilate more zinc, cadmium and copper than millipedes from the uncontaminated site when both populations are fed on contaminated litter (e.g. cadmium, fig. 6). This difference between the populations is one of the many topics which needs to be investigated further.

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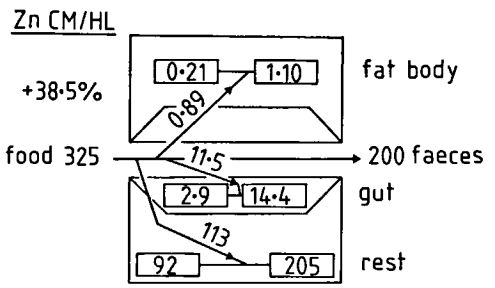

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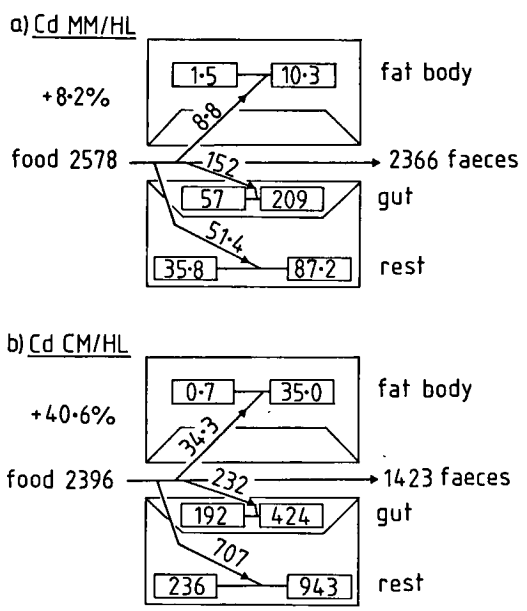

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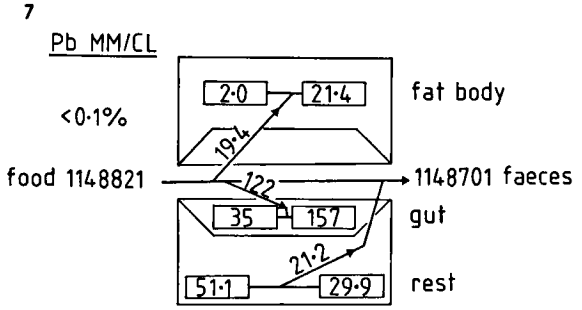

CONCLUSIONS AND SUGGESTED TOPICS FOR FURTHER RESEARCH

The ability to regulate the assimilation of essential and non-essential metals may be the major selective pressure in polluted sites that determines which species of centipedes and millipedes are able to survive and reproduce. It is therefore essential that more extensive studies are carried out into the dynamics of metal uptake and excretion in myriapods. Some of the lines of research which might be profitable to follow are outlined below.

1. A detailed survey of the concentrations of metals in a range of species of centipedes and millipedes from polluted and unpolluted sites. Some contaminated areas may have an unusual fauna as a result of differential resistance of different species to pollutants.

2. Ultrastructural studies on the ways in which the cells of different organs, particularly the gut, deal with metals.

3. Breeding experiments to determine whether the differences in the extent to which different populations of *Lithobius variegatus* and *Glomeris*

Figs. 5-7. Estimated assimilation rates (%) and net fluxes in amounts of metals through *Glomeris marginata* and between the 'gut', 'fat body' and 'rest' tissue fractions between the beginning and end of the experiment. The value given in the left hand box of each tissue fraction represents the amount of metal estimated to be present at the start of the experiment. The value in the right hand box represents the amount present at the end. 5: Zinc (nmol) in Charterhouse millipedes fed on Haw litter (CM/HL). 6a: Cadmium (pmol) in Midger millipedes fed on Haw litter (MM/HL). 6b: Cadmium (pmol) in Charterhouse millipedes fed on Haw litter (CM/HL). 7: Lead (pmol) in Midger millipedes fed on Charterhouse litter (MM/CL).

marginata assimilate heavy metals are due to acclimation of physiological processes during the lifetime of the animal, or to the evolution of mechanisms which can be genetically inherited.

ACKNOWLEDGEMENT

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