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Heavy Metals in the Centipede *Lithobius variegatus* (Chilopoda)

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ABSTRACT

The distribution of zinc, cadmium, lead and copper was determined in the tissues of the centipede Lithobius variegatus *(Leach) collected from two uncontaminated deciduous woodlands and two sites close to a smelting works. Centipedes from all four sites contained large amounts of zinc which are stored primarily in the fat body and tissues associated with the exoskeleton. Cadmium, lead and copper were present in much smaller amounts than zinc and are contained mostly in the midgut.*

Concentrations of copper were much higher in the midguts of centipedes from near the smelting works than in animals from the isolated rural woodlands. In contrast, levels of zinc, cadmium and lead in L. variegatus *showed little correlation with the degree of contamination of the site from which they were collected.*

INTRODUCTION

Recent research on saprophagous invertebrates collected from sites polluted by smelting or mining operations, or automobile exhausts, has shown that earthworms (Ireland, 1975), Collembola (Joosse & Buker, 1979), snails (Coughtrey & Martin, 1976) and woodlice (Hopkin & Martin, 1982*a*) may accumulate considerable amounts of heavy metals. These authors have suggested that the high concentrations of metals in these animals may have detrimental effects on predators. However, although there have been a number of studies on predatory vertebrates (e.g. Roberts *et al.*, 1978; Avery *et al.*, in press), there has been very little work carried out on the effects of metals on carnivorous invertebrates such as centipedes.

In temperate deciduous woodlands lithobiid centipedes may occur in densities of up to $50\,m^{-2}$ and, in such sites, are relatively of greater importance as predators than other invertebrate species (Wignarajah & Phillipson, 1977). In this study, the concentrations of zinc, cadmium, lead and copper have been determined in *Lithobius variegatus* collected from four woodlands in South West England. Two of the sites are in isolated rural areas and two are close to a primary smelting works and are heavily contaminated with zinc, cadmium, lead and copper. The results presented in this paper are, as far as is known, the first to be published on the concentrations of heavy metals in Chilopoda.

MATERIALS AND METHODS

Specimens of adult *Lithobius variegatus* were collected from Midger Wood (British Ordnance Survey grid reference ST 796 893), Wetmoor Wood (ST 743 876), Haw Wood (ST 560 798) and Hallen Wood (ST 555 802) during April 1982. Midger and Wetmoor are uncontaminated sites whereas Haw and Hallen are 3 km downwind of a primary smelting works and are heavily contaminated with zinc, cadmium, lead and copper (Hopkin & Martin, 1982*a*; Martin *et al.*, 1982).

The centipedes were starved for 4 days in individual Petri dishes to allow the contents of the gut to be voided. At the end of this period, seven centipedes of 2 to 3 cm in length were selected from each site population and the tissues dissected into ten fractions. Soft tissues were placed on small pieces of Millepore filter paper which had been dried and weighed. A longitudinal cut was made along the full length of the ventral surface and the Malpighian tubules (tissue fraction 1), oesophageal glands (2), reproductive organs (3), midgut (4) and nerve cord (5) were removed. The legs (6), poison claws (7) and head (8) were separated from the body. Finally, the fat body and connective tissue adhering to the cuticle was scraped from the inner surface of the body wall (9, the 'sub-cuticular tissue'). The remains (10, 'exoskeleton') consisted of the cuticle of the body wall and tissues which were too firmly bound to be removed by scraping. In addition, the exuvia of three centipedes which had moulted

TABLE 1
Tissue Dry Weight (TDW) and Concentrations of Zinc, Cadmium, Lead and Copper in *Lithobius variegatus* from Four Sites ($n = 7$ for each site)

	TDW (mg)	*Concentration ($\mu g\, g^{-1}$ dry weight)*			
		Zn	*Cd*	*Pb*	*Cu*
Midger					
NC	0·241 ± 0·038	163	<0·6	<3·0	42·0
MT	0·165 ± 0·025	137	<0·9	<4·3	100
OG	0·155 ± 0·013	200	<0·9	<4·6	199
RO	1·723 ± 0·312	93	<0·08	<0·4	9·5
PC	0·614 ± 0·072	84	<0·2	<1·2	21·0
Head	1·735 ± 0·155	155	<0·08	<0·4	11·4
Legs	3·005 ± 0·110	110	<0·05	<0·2	1·77
MG	1·670 ± 0·204	658 ± 35	3·23 ± 1·11	<3·0	61·4 ± 7·2
SCT	0·457 ± 0·090	1 840 ± 431	<1·5	<11	61·4 ± 9·5
EXO	10·212 ± 0·540	439 ± 38	<0·05	<0·5	8·3 ± 1·0
Mean	19·977 ± 2·72	367	<0·42	<1·0	16·4
Wetmoor					
NC	0·199 ± 0·029	122	<0·7	<3·6	62
MT	0·144 ± 0·022	114	<1·0	<5·0	123
OG	0·127 ± 0·013	210	<1·1	<5·6	234
RO	1·989 ± 0·348	84	<0·07	<0·4	11·6
PC	0·462 ± 0·039	112	<0·31	<1·5	47·0
Head	1·229 ± 0·083	137	<0·12	<0·6	7·30
Legs	2·825 ± 0·169	90	<0·05	<0·2	1·65
MG	1·382 ± 0·165	431 ± 23	5·29 ± 0·97	<3·5	45·3 ± 9·6
SCT	0·307 ± 0·049	932 ± 205	<3·5	<16	49·1 ± 10·8
EXO	7·082 ± 0·296	292 ± 29	<0·14	<0·7	10·3 ± 1·4
Mean	15·713 ± 0·892	233	<0·66	<1·3	25·7
Haw					
NC	0·279 ± 0·041	151	<0·5	<2·5	10·3
MT	0·202 ± 0·034	180	<0·7	<3·5	42·0
OG	0·206 ± 0·034	192	<0·7	<3·5	211
RO	2·297 ± 0·421	100	<0·06	<0·3	11·3
PC	1·063 ± 0·252	111	3·10	<0·7	15·7
Head	2·541 ± 0·202	194	4·23	<0·3	17·9
Legs	3·365 ± 0·994	119	1·05	<0·2	7·53
MG	2·050 ± 0·336	764 ± 54	26·8 ± 12·9	28·1 ± 4·1	206 ± 53
SCT	1·120 ± 0·130	3 074 ± 983	<0·9	<4·5	58·3 ± 10·1
EXO	11·782 ± 0·707	454 ± 48	0·85 ± 0·19	<0·4	11·3 ± 1·60
Mean	24·904 ± 4·399	470	<3·4	<2·9	31·7

(*continued*)

TABLE 1—*contd.*

	TDW (mg)	*Concentration ($\mu g\ g^{-1}$ dry weight)*			
		Zn	*Cd*	*Pb*	*Cu*
Hallen					
NC	0·201 ± 0·035	172	<0·7	<3·5	6·41
MT	0·153 ± 0·028	179	<0·9	<4·7	55·3
OG	0·148 ± 0·024	212	<1·0	<4·8	284
RO	1·795 ± 0·311	87·4	<0·08	<0·4	17·5
PC	0·707 ± 0·080	105	1·91	<1·0	15·7
Head	1·922 ± 0·288	210	4·26	<0·4	27·2
Legs	2·114 ± 0·402	144	2·75	<0·3	5·38
MG	1·950 ± 0·341	857 ± 62	85·0 ± 36·0	12·3 ± 6·1	398 ± 95
SCT	1·212 ± 0·174	2 450 ± 754	<0·8	<4·2	82·6 ± 7·8
EXO	7·994 ± 0·631	612 ± 55	1·14 ± 0·22	<0·5	9·4 ± 1·10
Mean	18·196 ± 2·910	581	<10·5	<2·1	60·9

The nerve cord (NC), Malpighian tubules (MT), oesophageal glands (OG), reproductive organs (RO), poison claws (PC), head and legs were pooled after weighing. Cadmium and lead were not detected in samples prefixed by <. These figures have been calculated on the assumption that the digests contained concentrations of the metals at just below the detection limit of the analytical equipment. All figures for tissue dry weights and concentrations of metals in the midgut (MG), subcuticular tissue (SCT) and exoskeleton (EXO) are means ± standard errors.

in the laboratory were collected for analysis. For a detailed description of the internal anatomy of centipedes, see Lewis (1981).

The tissues were placed in glass Petri dishes and dried overnight at 70 °C. After cooling to room temperature, the samples were weighed on a microbalance. A few crystals of silica gel were placed in each Petri dish and in the weighing chamber of the microbalance to prevent fluctuations in the moisture content of the tissues.

The material was digested in boiling concentrated Aristar grade nitric acid (BDH Chemicals, Poole, Dorset, UK) and diluted to 5 ml with deionised distilled water. Blank digests of Millepore filter paper were also prepared. The digests were analysed for zinc, cadmium, lead and copper by flame (Varian AA775) or flameless (Varian AA6 and CRA90) atomic absorption spectrophotometry (AAS). In all cases, correction for non-atomic absorption was made automatically with a deuterium or hydrogen lamp, respectively.

Analyses of individual samples of tissues could be performed only on the midgut, sub-cuticular tissue and exoskeleton. The concentrations of metals in the other seven fractions were, in most cases, below the detection limit of the spectrophotometers and the samples of each tissue had to be pooled.

With flameless AAS, concentrations of 0·000 2 $\mu g\,ml^{-1}$ of cadmium and 0·001 $\mu g\,ml^{-1}$ of lead could be detected in the digests. However, these elements could not be detected in many tissue fractions and figures in Table 1 and Fig. 1 prefixed by < have been calculated on the assumption

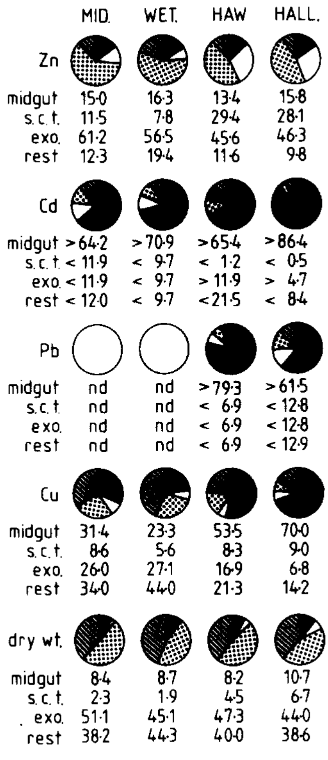

Fig. 1. Mean percentage distribution of zinc, cadmium, lead and copper and dry weight between the midgut, sub-cuticular tissue (s.c.t.), exoskeleton (exo.) and rest of the body tissues of *Lithobius variegatus* from four sites. Solid shading, midgut; unshaded, sub-cuticular tissue; stippled shading, exoskeleton; lined shading, rest of tissues. nd, not detected in any samples from the site. Cadmium or lead were detected in tissues where figures are prefixed by >. In cases where cadmium or lead were not detected, the percentage distributions have been calculated on the assumption that metals were present at just below the detection limit of the analytical equipment. Thus, figures prefixed by < or > represent maximum or minimum theoretical values, respectively.

that the digests contained concentrations of cadmium or lead which were just below the detection limits of the analytical equipment. In most cases these are probably an overestimate but they represent a maximum value which can be compared with other tissues in which the metals could be detected.

RESULTS

There were some marked differences between the concentrations of zinc, cadmium, lead and copper in the tissue fractions of centipedes from all four sites (Table 1). Concentrations of zinc were particularly high in the midgut, exoskeleton and sub-cuticular tissues. In contrast, the centipedes contained relatively small amounts of copper and very low levels of cadmium and lead. These three elements are stored mainly in the midgut.

The mean concentrations of zinc, cadmium, lead and copper were, in general, higher in the tissues of centipedes from Haw and Hallen than in those from Midger and Wetmoor. However, few of these differences are statistically significant (Table 2) due to the relatively small number of animals dissected and the large individual variation. Indeed, the only case where concentrations of a metal were significantly higher in both Haw and Hallen centipedes than in Midger and Wetmoor animals is for copper in the midgut (Table 2).

Analyses of the distribution of amounts of metals between the tissues confirm that zinc is stored primarily in the sub-cuticular fraction and exoskeleton whereas cadmium and copper, and lead when detectable, occur mainly in the midgut (Fig. 1). The differences in the relative amounts of metals stored by each tissue fraction are particularly apparent in centipedes from contaminated sites. For example, in animals from Hallen, the sub-cuticular tissues comprised a mean of only 6·7 % of the dry weight but contained a mean of 28·1 % of the zinc in the whole animal. Furthermore, the midgut comprised a mean of only 10·7 % of the dry weight but contained a mean of 70·0 % of the copper, more than 86·4 % of the cadmium and greater than 61·5 % of the lead in the whole animal.

Zinc, cadmium, lead or copper were not detected in the exuvia of centipedes which had moulted in the laboratory. Therefore, the large amounts of zinc in the exoskeleton fractions are probably associated with living tissues underlying the cuticle. Centipedes invariably eat the cast exoskeleton soon after moulting so deposition in the cuticle before ecdysis

TABLE 2

Results[a] of Student *t* Tests to Determine Statistical Significances of Differences Between the Mean Concentrations of Zinc, Cadmium, Lead and Copper in Tissues of *Lithobius variegatus* from Four Sites

	Midger	*Wetmoor*	*Haw*	*Hallen*
Zinc—Sub-cuticular Tissues				
Midger	×	$1{\cdot}901^{ns}$	$1{\cdot}153^{ns}$	$0{\cdot}702^{ns}$
Wetmoor	×	×	$2{\cdot}133^{ns}$	$1{\cdot}943^{ns}$
Haw	×	×	×	$0{\cdot}503^{ns}$
Hallen	×	×	×	×
Zinc—Midgut				
Midger	×	5·420***	$1{\cdot}654^{ns}$	2·795*
Wetmoor	×	×	5·676***	6·443***
Haw	×	×	×	$1{\cdot}137^{ns}$
Hallen	×	×	×	×
Zinc—Exoskeleton				
Midger	×	3·071**	$0{\cdot}245^{ns}$	2·587*
Wetmoor	×	×	2·882*	5·145***
Haw	×	×	×	$2{\cdot}163^{ns}$
Hallen	×	×	×	×
Cadmium—Midgut				
Midger	×	$1{\cdot}397^{ns}$	$1{\cdot}829^{ns}$	2·270*
Wetmoor	×	×	$1{\cdot}664^{ns}$	2·213*
Haw	×	×	×	$1{\cdot}500^{ns}$
Hallen	×	×	×	×
Cadmium—Exoskeleton				
Haw	×	$0{\cdot}995^{ns}$		
Hallen	×	×		
Lead—Midgut				
Haw	×	$0{\cdot}995^{ns}$		
Hallen	×	×		
Copper—Sub-cuticular tissues				
Midger	×	$0{\cdot}852^{ns}$	$0{\cdot}222^{ns}$	$1{\cdot}729^{ns}$
Wetmoor	×	×	$0{\cdot}623^{ns}$	2·514*
Haw	×	×	×	$1{\cdot}903^{ns}$
Hallen	×	×	×	×
Copper—Midgut				
Midger	×	$1{\cdot}341^{ns}$	2·703*	3·533**
Wetmoor	×	×	2·987*	3·690**
Haw	×	×	×	$1{\cdot}765^{ns}$
Hallen	×	×	×	×
Copper—Exoskeleton				
Midger	×	$1{\cdot}139^{ns}$	$1{\cdot}565^{ns}$	$0{\cdot}714^{ns}$
Wetmoor	×	×	$0{\cdot}476^{ns}$	$0{\cdot}508^{ns}$
Haw	×	×	×	$0{\cdot}478^{ns}$
Hallen	×	×	×	×

[a] The figures in the tables are values of *t* together with the level of significance. ns, Not significant; *, 5 %; **, 1 %; ***, 0·1 % (12 degrees of freedom).

would not be an effective method of permanently removing potentially harmful substances from the body.

The oesophageal glands and sub-cuticular tissues of centipedes from all four sites contained relatively high concentrations of copper (Table 1). Much of this metal may be bound in lithobioviolin, a striking violet pigment based on copper (Needham, 1960), which is present in large amounts in these tissues (Lewis, 1981).

DISCUSSION

A large proportion of the diet of lithobiid centipedes consists of Collembola and woodlice (Roberts, 1956; Sunderland & Sutton, 1980). In sites contaminated by heavy metals, woodlice contain much higher concentrations of zinc, cadmium, lead and copper (Hopkin & Martin, 1982*a*) and Collembola much higher concentrations of lead (Joosse & Buker, 1979) than the same species from uncontaminated sites. Therefore, it is surprising that, with the exception of copper in the midgut, differences in the concentrations of metals in the tissues of *Lithobius variegatus* show little correlation with the degree of contamination of the site from which they were collected.

There are two possible reasons why the concentrations of zinc, cadmium and lead were similar in *L. variegatus* from uncontaminated and contaminated sites. First, these metals may be bound in the prey in a form which is insoluble in the digestive fluids. Secondly, centipedes may be able to regulate the intake of zinc and prevent the uptake of non-essential elements, such as cadmium and lead, even if these metals are present in very high concentrations in their food. Woodlice and Collembola store heavy metals in highly insoluble intracellular granules in digestive tissues (Humbert, 1978; Hopkin & Martin, 1982*b*). Thus, much of the metal which enters the centipedes in the food may remain bound in this form in the lumen of the gut and be voided in the faeces. However, if these granules are digested and the metals released into solution, then efficient mechanisms must have evolved to limit the uptake of zinc, cadmium and lead from the food. Indeed, centipedes would be expected to have evolved such systems because the amounts of these elements in their prey in uncontaminated sites are relatively large (Hopkin & Martin, 1982*a*).

Copper is required by centipedes for the production of respiratory proteins such as haemocyanin in the blood (Rajulu, 1969) and lithobioviolin in the tissues surrounding the trachea (Needham, 1960). At Hallen and Haw, where levels of this element in the prey are very high, the centipedes may not be able to prevent uptake of copper in excess of their requirements and the concentrations in the midgut are consequently greater. However, the concentrations of zinc in *L. variegatus* are much higher than those of copper at all four sites. Presumably, zinc is involved in an essential biochemical process in centipedes which has yet to be described.

Studies are under way into the assimilation of heavy metals by centipedes in the laboratory and the ultrastructural distribution of these elements in the tissues.

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