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Biological Half-lives of Lead in *Orchesella cincta* (L.) (Collembola)

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The organic soil horizon is a major sink of heavy metals deposited in forest areas (Friedland *et al.* 1984). The persistence and gradual accumulation of toxic metals in soils have raised concern on the possible irreversible impairment of ecological soil processes (Babich and Stotzky 1985). Multifunctionality of the soil system is one of the main principles underlying soil protective legislation in the Netherlands, and is endangered by irreversible change.

Many invertebrate animals are active in normal and contaminated soil; they all cycle contaminants from the soil through their bodies and in doing so may change the binding of metals. Solubility of zinc and calcium in soil was changed after passage through the guts of earthworms (Ireland 1975). A similar effect on copper was observed by Dallinger and Wieser (1977) in isopods. To quantify the possible long-term changes, brought about by soil animal activity, information is needed on the residence-times of metals in different soil invertebrates. Compartment theory is considered as the appropriate approach in these types of problems (Moriarty 1978), and is applied in this paper.

Compartment models for distribution of heavy metals within organisms have been developed most extensively for man (Nordberg and Kjellström 1979; Marcus 1979). Analysis of heavy metal turnover by arthropods has been done using radioactive tracers (Van Hook and Yates 1975; Webster and Crossley 1978; Giesy *et al.* 1980). Others have determined the distribution of heavy metals over various organs of the invertebrate body, and from it, deduced the cycling pathways (Andersen and Laursen 1982; Hopkin and Martin 1984; Prosi and Back 1985). Our results show that useful information on kinetics can also be obtained by direct analysis of the metal in an accumulation-elimination experiment. It appears that, even in such small animals as Collembola, different compartments can be recognized, each with different residence times of lead.

Collembola (Insecta Apterygota) are among the most numerous of forest soil invertebrates, especially in mor-type profiles. Because of their high abundance and productivity, these insects are considered as important links in biomass transfer in the forest soil (Gist and Crossley 1975). Whether they also transfer significant amounts of pollutants depends on the pertinent mechanisms of assimilation and excretion of these substances. For a forest floor population of the species *Orchesella cincta*, it was calculated in van Straalen *et al.* (1985) that the food-chain transfer of lead was very minor (about 11 times the population's standing pool per year), compared to the flux of lead through consumption and defaecation (10,000 times the standing pool per year). Given the relatively high resistance of Collembola to lead (Joosse and Buker 1979; Joosse and

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Verhoef 1983), the effects they have on cycling of metals may be more important than the effects they suffer from intoxication.

In this paper we report on experimental results on the biological half-lives of lead for different compartments of *Orchesella cincta*. This may contribute to understanding both the physiological kinetics of lead, underlying resistance, as well as the cycling of lead through field populations.

MATERIALS AND METHODS

About 100 adult Collembola of the species *Orchesella cincta* (L.) were collected from an unpolluted pine forest at Dronten, the Netherlands. These animals were cultured in the laboratory at 20°C for several weeks on unpolluted twigs overgrown with green algae. The offspring were separated from their parents and raised in the same way until nearly adult. Only the F_1 -generation was used in the experiments.

In the main experiment, the experimental unit was a PVC container (Ø 16 cm), fit with a plaster of Paris bottom and covered with a gauze lid. A layer of broken cocktail sticks served as shelter for the collembolans. Food was offered as a thick suspension of green algae (*Pleurococcus* spp.) from the bark of trees, on ten paper discs per container. Containers were cleaned and food was refreshed every week. Contaminated food was prepared by adding an amount of lead nitrate solution to the food suspension, in such a way that a nominal concentration of 2000 µg Pb/g dry weight of food was obtained. The actual concentration measured afterwards varied between different food preparations from 1600 to 2200 µg/g.

Five containers were observed, each with 200 animals at the beginning. At 21 occasions during the experiment, the lead concentration of the animals was monitored by taking four individuals from each container. Two were sacrificied immediately and stored frozen; the other two were placed in an empty container for one night to discharge their gut contents. All collembolans were dried, weighed and analysed for lead by atomic absorption as described in van Straalen and van Wensem (1986). By pooling two individuals from each container, we were sure that even the lowest concentrations could be measured reliably. Food and faeces were analysed for both total and exchangable lead; the latter was estimated by vigorously shaking 10 mg food or faeces with 0.5 ml 1 M NH_4Ac , pH 7 for two hours. The experiment lasted for eight weeks. During the first period of four weeks the food was contaminated, while in the second period clean food was offered.

In a separate experiment, the effect of gut emptying on lead body burden was studied. Collembola that had accumulated lead before, were placed in seven containers without food. Each container had 15 individuals. At times 0, 2, 4, 7, 9, 12 and 23 hours after the beginning of the experiment, collembolans from one of the containers were sacrificied; five of them were frozen for lead analysis, ten were preserved for inspection of gut contents. Filling of the gut was estimated as a percentage of the total digestive tract, for each individual, following clearing in 10% KOH.

Elimination kinetics were described by a three compartment linear model. An equation of the form $C = C_0 \exp(-kt)$ was fitted to the tail of the data from the main experiment and then, after "peeling off" the tail, to the rest. Data from the gut emptying experiment were treated separately and were fitted by a single exponential. From the estimate for k ($\hat{k}$), the biological half-life (T) was estimated from $\hat{T} = (\ln 2)/\hat{k}$. Confidence limits for $\hat{T}$ were estimated from confidence limits for $\hat{k}$, which were based on regression analysis.

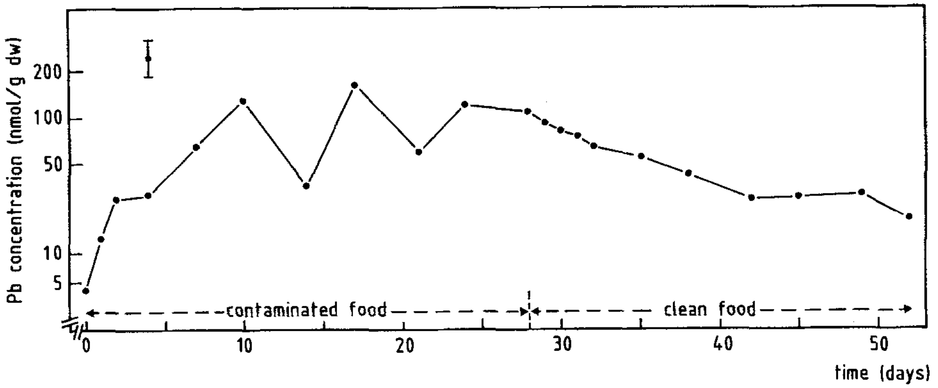

Figure 1. Lead concentration of *Orchesella cincta* during the accumulation-elimination experiment, excluding gut contents. Note the logarithmic scale. Each mean is based on five replicate observations; each observation is based on analysis of two pooled individuals. Each mean has a standard error as shown in the left upper corner, which was derived from the analysis of variance error mean square. Only the data for defaecated animals are presented.

RESULTS AND DISCUSSION

Lead concentrations of *Orchesella cincta* during the accumulation-elimination experiment were subjected to analysis of variance following logarithmic transformation of the data. From this analysis it was concluded that defaecation significantly lowered the lead concentration, especially during the accumulation period of the experiment. Averaged over the first period, 48% of the body burden can be attributed to contents of the digestive tract.

The results from the accumulation-elimination experiment are presented in Fig. 1. It is clear that lead concentrations fluctuate within wide limits during the accumulation phase, even when the collembolans have emptied there guts. An average steady state is achieved after approximately four weeks. During the elimination phase, however, concentrations behave more smoothly. They approach a level which, even after four weeks, is still higher than the concentration at the beginning. Apparently, part of the accumulated burden cannot be excreted anymore, or at least at a very slow rate. The concentration reached after four weeks of depuration is 31% of the concentration at the end of the accumulation phase.

Emptying of the gut is completed within one day (Fig. 2). In the beginning, individuals with empty and with filled guts are present both. This variation is due to the moulting cycle: in an asynchronous population of *Orchesella cincta* about 50% has empty guts because of preparations for moulting (Testerink, 1981). Gut emptying is accompanied by a discharge of about 50% of the lead body burden (Fig. 3), which is in good agreement with the results of the main experiment.

On the basis of the data presented above we concluded that lead in *Orchesella cincta* is present in three compartments, each having different turnover rates: (i) the digestive tract, (ii) a "fast body burden", (iii) a "slow body burden". Next we estimated the half-lives of lead in each of the compartments, and the share of each compartment in the total burden achieved at the end of the accumulation period. Table 1 shows that the most important compartment, both in size and in turnover, is the digestive tract. Lead in the gut

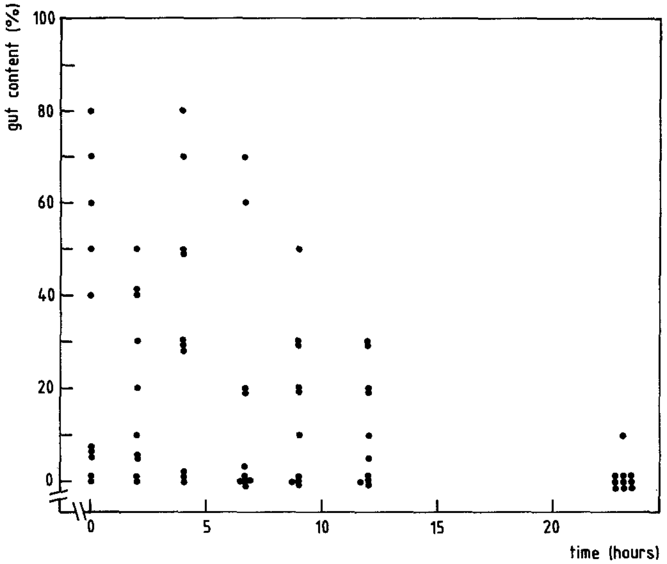

Figure 2. Gut contents of individual *Orchesella cincta* during the food deprivation experiment. The filling is related to the maximal content of the digestive tract, and is expressed as a percentage.

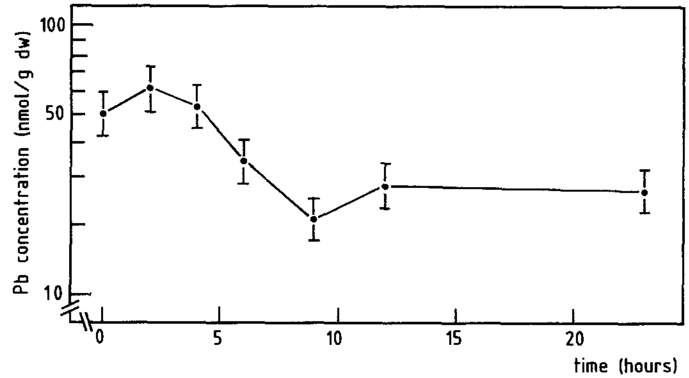

Figure 3. Lead concentrations of individual *Orchesella cincta* during the food deprivation experiment. Each mean is based on five replicate observations. Standard errors were derived from the analysis of variance error mean square.

has a half-life of less than a day; the fast body burden only halves within a week, while the slow body burden takes about three weeks. Confidence intervals for the estimates are rather wide, due to the relatively great variation between individuals.

Because most of the lead consumed passes rapidly through the digestive tract, we investigated whether this changed the binding of lead in the food. Fig. 4 shows that extractability of lead in faeces is consistently higher than in food. It also decreases during the experiment, possibly due to aging of the food stock (although every week a fresh preparation from the stock was made). It may be concluded that lead, bound to green

Table 1. Estimates for rate constants and half-lives for lead in *Orchesella cincta*.

compartment	rate constant (days^{-1})	half-life (days)	% of total burden
gut	2.02 0.68 - 3.35	0.34 0.21 - 1.02	48
fast body burden	0.094 0.058 - 0.130	7.37 5.33 - 11.95	36
slow body burden	0.032 - 0.021 - 0.085	21.66 8.15 - ∞	16

Below each estimate a 95% confidence interval is given.

algae amended with lead nitrate, becomes more soluble in passing the digestive tract of *Orchesella cincta*. The ecological relevance of the compartmented behaviour of lead can be indicated as follows:

(i) The digestive tract represents half of the body burden and has a very rapid turnover. The binding of lead in food is changed while it passes through the gut. This has also been observed for zinc and calcium (but not for lead) in earthworms (Ireland 1975) and for copper in isopods (Dallinger and Wieser 1977). Further experiments are in progress to assess whether different metals are solubilized to different degrees by Collembola. The effect of gut passage is not realized by means of low intestinal pH conditions; Humbert (1974) has shown that the intestinal pH of the collembolan *Sinella coeca* is mildly acidic to alkaline (5.4-8.8). The effect of gut passage possibly relates to the fact that assimilation efficiency of lead in *Orchesella cincta* is only 0.4% (van Straalen *et al*. 1985), while for the food as a whole it is 22% (Testerink 1982). Given the large amounts of lead processed by an *Orchesella cincta* population (van Straalen *et al.* 1985), it may be concluded that Collembola will have a long term solubilizing effect on lead in the soil.

(ii) The second compartment, called "fast body burden", probably represents the fraction of lead mobilizable by the excretion mechanism of intestinal exfoliation (Joosse and Buker 1979). On the basis of the rate constant estimated for this compartment (Table 1), it can be calculated that 42% of the body burden is excreted by this mechanism per moulting interval (mean instar duration at 20°C = 5.8 days: Joosse and Veltkamp 1970). This is in good agreement with estimates of excretion efficiency from budget experiments, which have yielded a mean of 43% per moulting interval (van Straalen *et al.* 1985).

(iii) The third compartment, called "slow body burden" is more difficult to interpret in physiological or morphological terms. It has a half-life approaching the life-time of Collembola in the field, and therefore represents a fraction which will accumulate during life. In an *Orchesella cincta* field population, older (larger) individuals have significantly higher burdens of lead than young individuals (van Straalen 1986). Therefore, it is this fraction that may exert a toxic effect, since it cannot be regulated adequately. Toxic effects of lead were observed by Joosse and Verhoef (1983) in *Orchesella cincta*, and by Bengtsson *et al.* 1983, 1985) in *Onychiurus armatus*. These effects find expression as a lowering of oxygen consumption and a decrease of growth, fertility and survival.

Finally, it may be noted that the compartment model may be useful as a means of comparing species (Moriarty 1978). Van Straalen and van Wensem (1986) observed that

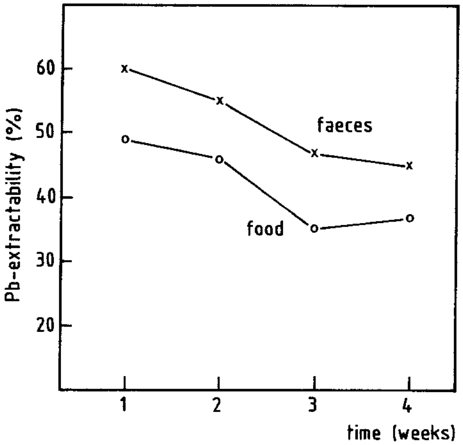

Figure 4. Extractability of lead in food and faeces during the accumulation period of the accumulation-elimination experiment. Extractability is based on 1 M NH Ac-extraction, relative to the total concentration (HNO /HClO -digestion).

the heavy metal content of forest soil arthropods significantly differed between species and did not seem to relate to the trophic level to which species belonged, nor to their body-size. A theory based on similarity of physiological equipment may be more promising in predicting the accumulating potential of species; such a theory could make use of the compartment model approach as a unifying formalism.

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