The effect of high lead concentrations on the mortality, mass and behaviour of *Porcellio laevis* Latr. (Crustacea, Isopoda) in laboratory tests

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The aim of this study was to determine the effects of high lead concentrations on the terrestrial isopod *Porcellio laevis* and to determine whether these animals could distinguish between lead contaminated and uncontaminated leaves. In the acute toxicity tests the isopods were exposed to 0, 15, 30, 45, 60, 75, 90 and 105 g/kg lead nitrate. Behavioural tests were conducted exposing the isopods to 0, 15 and 105 g/kg lead nitrate. In the acute toxicity tests the various concentrations differed significantly concerning mortality and weight loss of surviving isopods. The LC_{50} after 14 days exposure for *P. laevis* was 87.4 g/kg. The LC_{50} values obtained in this study are extremely high compared to those of earthworms. This is an indication of high tolerance for lead. The concentrations of lead found in the isopods were high and could give a possible explanation for the mortalities and mass losses observed, as lead at those concentrations could have disturbed the normal physiological functioning of the animals. The isopods avoided lead contaminated leaves in the behavioural tests, which could cause accumulation of leaf litter and thus a reduction of the decomposition rate.

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In today's industrialised society there is a continued threat of metal pollution to organisms. The deposition of metals from the atmosphere and resulting accumulation in terrestrial ecosystems is a well-known phenomenon (Tyler 1972). It is also known that certain metals damage various organisms. On an ecosystem level, pollution of soils can arise from aerial deposition from sources such as smelting works or car exhausts, or from persistence of contamination following closure of metalliferous mines (Hopkin 1994). A significant amount of lead enters the atmosphere owing to the combustion of lead-enriched petrol and is consequently deposited in the soil, entering terrestrial ecosystems. The soil-litter component of the terrestrial ecosystem is an important sink for metal contaminants (Coughtrey, Jones, Martin & Shales 1979). The concentration of metals is increasing faster in the soil-litter than in other parts of the ecosystem (Tyler 1972, 1984). Terrestrial isopods, like many other soil invertebrates, occur in this zone. The normal functioning of the ecosystem depends on decomposition for its supply of several important nutrients. Terrestrial isopods play an important role in the decomposition process (Hassall, Turner & Rands 1987). According to Hopkin (1994) one of the characteristic features of metal-contaminated ecosystems is a reduction in the rate of decomposition of dead plant material. Lead is one of the metals that reduces the rate of decomposition and its effect may persist for several years (Doelman & Haanstra 1979). If terrestrial isopods are detrimentally affected by lead, it could lead to a decrease in decomposition rate in lead contaminated leaf litter.

The aim of this study was to determine the effects of high lead concentrations, administered in the form of lead nitrate, on the terrestrial isopod *Porcellio laevis*, and to determine whether these animals could distinguish between lead contaminated and uncontaminated leaves.

Materials and methods

The terrestrial isopod *Porcellio laevis*, which occurs abundantly in leaf litter in the south-western Cape in South Africa, was used in this study. Tests were conducted using specimens at least 12 mm in length. Since isopods have a fairly cosmopolitan diet of fungal hyphae, leaf litter and other plant material (Hopkin 1991), decaying oak leaves, collected from the Botanical Gardens of the University of Stellenbosch, were chosen as food and route of contamination. All experiments were conducted in a climate-controlled room with a temperature of 16°C (± 2°C) and in darkness.

Acute toxicity tests

Round containers 11 cm in diameter with a moistened plaster of Paris bottom (Joosse, Wulffraat & Glas 1981) were used in these tests. Oak leaves to be used in the tests were kept moist, before the commencement of the tests, in closed plastic bags to ensure that the microorganisms flourished and did not disappear. Leaves were shredded to small pieces in a food processor, after which they were spread out on a flat surface to dry at room temperature. 50 g dry mass of leaves was added to each of the containers used. Lead was administered in the form of lead nitrate [$Pb(NO_3)_2$]. At first, range-finding tests were performed to determine the concentrations to be used in the tests. Concentrations to be used were determined to be 0, 15, 30, 45, 60, 75, 90 and 105 g lead nitrate per kg dry mass leaves. A calculated mass of lead nitrate was weighed for each desired concentration and each was dissolved in 20 ml distilled water. The solutions of lead nitrate were sprayed on the leaves (50 g dry mass) to give the desired concentrations. Six replicates of each of the concentrations were prepared. Ten isopods were placed in each of the test containers. Before the start of the tests, they were weighed and during the tests they were also weighed after 7 and 14 days. Mortality was also checked after 7 and 14 days in each of the containers. After the 14-day period a single isopod was collected from each container. They were starved for 24 h (Hames & Hopkin 1989) to rid the gut of any food. They were killed with chloroform and freshly weighed before being temporarily stored

in glass vials in a freezer. The metal content of the isopods which died during the experiment was not determined.

The following process was used for the acid digestion of the isopods. 2 ml 55% nitric acid was added to each individual sample and left overnight. They were then heated to 40°C for 1 h. This was followed by 45 min of 120°C heat. A blank digestion was also performed with each of the sets of digestions to keep a check on possible contamination. Each of the samples was filtered through a 0.45 µm Millipore filter and made up to 10 ml with distilled water. Analysis for lead was performed with a Varian AA-1275 atomic absorption spectrophotometer. The lead concentrations in the isopods were expressed on a wet mass basis. Twelve isopods were oven dried at 60°C for 24 h. The percentage mass loss was calculated to be 82.32% (*S.D.* ± 2.30). This conversion factor was used to express the lead concentrations in the isopods on a dry mass basis. Leaves were sampled from each of the test containers and were dried in a drying oven at 60°C for 48 h. The samples were then ground to a powder and digested in the following way. 10 ml 55% nitric acid was added to each sample and left overnight. They were heated to 40°C for 1 h and then to 120°C for 3 h. After digestion the samples had a whitish coloured sludge, which had to be removed by filtration. The samples were centrifuged and filtered, after which they were made up to 400 ml to make the high concentrations used detectable for the atomic absorption spectrophotometer. Lead concentrations found in the leaves used in these tests were in all instances within 5% of the desired concentrations.

Behavioural tests

The tests were designed to determine whether *P. laevis* could distinguish between uncontaminated leaves and contaminated leaves and if they consequently avoided the contaminated leaves. Lead nitrate concentrations used were 0, 15 and 105 g lead nitrate per kg dry mass leaves. Leaves for these tests were prepared as decribed above. Twenty replicates of each experiment were conducted.

The containers used in these tests had dimensions of 16 x 10 x 7 cm and each was divided into an uncontaminated half (A) and a contaminated half (B). There was no physical division between sides (A) and (B). Side (A) contained leaves sprayed with distilled water and side (B) contained the leaves sprayed with the metal solutions. Before commencement of the tests the woodlice were starved for 24 h to rid the gut of food, therefore forcing the animals to make a choice. A single isopod was placed in each container to eliminate the aggregation phenomenon that may occur if a number of isopods are placed in each container (Takeda 1984). This could give a false picture of preference. The isopod of each container was placed right in the middle of the container on the border between the two sides. The isopods had a free choice between side (A) and side (B). Preferences were checked every hour for 24 h. After each observation the isopods were replaced in the middle of the containers so that they could 'choose' again. To eliminate the possibility of a false picture, a control was run where both sides (A) and (B) had uncontaminated leaves. To keep a check on tactism (Fraenkel & Gunn 1961), the containers were kept facing in the same direction right through all the experiments, including those of the control tests.

After each of the experiments, leaves from side (A) and side (B) were sampled for acid digestion. Samples were dried in a drying oven at 60°C for 48 h. The samples were then ground to a powder and digested, centrifuged and filtered using the same procedure described previously. Blank digestions were also performed. The samples were analysed for lead with a Varian AA-1275 atomic absorption spectrophotometer. Concentrations in the leaves used in the behavioural tests were within 5% of the desired concentrations.

Statistical analysis

Data were analysed using Jandel Scientific's Sigmastat 2.0 software package. Data of the acute toxicity tests was analysed with the Kruskal-Wallis One Way ANOVA on Ranks. Dunnet's method was used to compare the groups exposed to lead nitrate in the acute toxicity tests with the control. Multiple pairwise comparisons of the data were done using the Student-Newman-Kuels method. The LC_{50} was calculated by Probit Analysis. The *t*-test was used to analyse data of the behavioural tests.

Results

Acute toxicity tests

Only the groups exposed to the three highest concentrations showed a statistically significant difference from the control. When mortality of isopods was measured after 7 and 14 days ($p < 0.05$) (Figure 1). Weight loss of surviving isopods after 7 and 14 days differed significantly from the control in all the exposure groups ($p < 0.05$) (Figure 2).

Multiple pairwise comparisons revealed that the lead content of isopods of the different exposure groups differed significantly in most of the comparisons except for the 90 vs 105 g/kg, 45 vs 75 g/kg, 60 vs 75 g/kg and 45 vs 60 g/kg comparisons ($p < 0.05$) (Table 1).

The LC_{50} after 14 days exposure for *P. laevis* was 87.4 g/kg.

Behavioural tests

Table 2 compares the preferences of isopods exposed to uncontaminated leaves and lead nitrate contaminated leaves. There was a statistically significant difference between A and B at both concentrations tested, concerning mean percentage

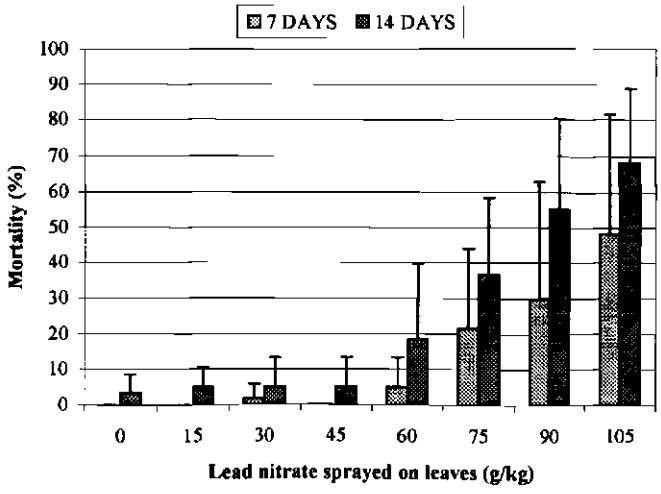

Figure 1 The percentage mortality (±S.D.) of *Porcellio laevis* at different exposure concentrations of lead nitrate after 7 and 14 days.

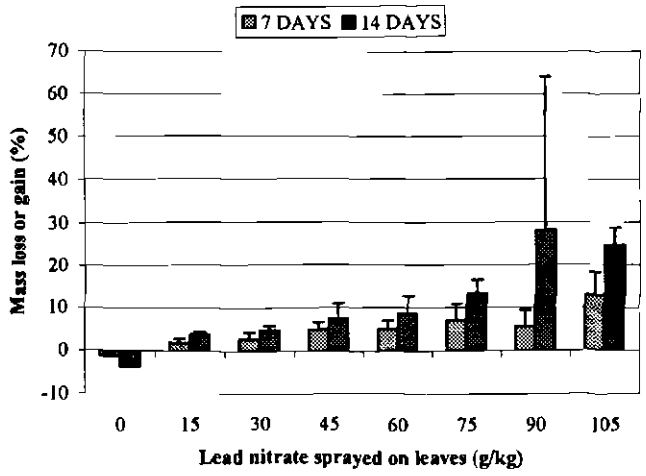

Figure 2 The percentage mass loss or gain (±S.D.) of *Porcellio laevis* after exposure for 7 and 14 days to different concentrations of lead nitrate. (Negative values indicate mass gain.)

Table 1 The lead concentrations (mg/kg) (wet and dry mass) in isopods after 14 days exposure to different concentrations (g/kg) of lead nitrate (± S.D). For each of the concentrations used $n = 6$. Significant difference from 0 = a, 15 = b, 30 = c, 45 = d, 60 = e, 75 = f, 90 = g, 105 = h ($p < 0.05$)

Exposure concentrations (g/kg)	Lead in isopods (mg/kg)	
	Wet mass	Dry mass
0	$0(\pm 0)^{bcdefgh}$	$0(\pm 0)^{bcdefgh}$
15	$65.0\ (\pm 7.0)^{acdefgh}$	$367.4\ (\pm 39.9)^{acdefgh}$
30	$173.6\ (\pm 76.2)^{abdefgh}$	$982.1\ (\pm 430.8)^{abdefgh}$
45	$238.3\ (\pm 68.8)^{abcgh}$	$1347.8\ (\pm 389.3)^{abcgh}$
60	$280.9\ (\pm 75.8)^{abcgh}$	$1588.6\ (\pm 428.8)^{abcgh}$
75	$295.5\ (\pm 78.0)^{abcgh}$	$1671.5\ (\pm 441.1)^{abcgh}$
90	$457.3\ (\pm 101.5)^{abcdef}$	$2586.4\ (\pm 574.3)^{abcdef}$
105	$599.6\ (\pm 263.7)^{abcdef}$	$3391.2\ (\pm 1491.5)^{abcdef}$

Table 2 Comparison of the preferences of *P. laevis* for uncontaminated leaves (A) and contaminated leaves (B) in behavioural tests. If no choice was exercised it was not taken into account in the calculations. For each of the concentrations used $n = 20$. #Significant difference between (A) and (B) ($p < 0.0001$)

Exposure concentrations (g/kg)	Uncontaminated leaves (A)		Contaminated leaves (B)	
	mean no. of choices (±SD)	mean % choices (±SD)	mean no. of choices (±SD)	mean % choices (±SD)
0	10.9 (±2.3)	53.0 (±13.6)	9.5 (±2.1)	47.0 (±3.6)
15#	15.9 (±2.5)	69.8 (±10.9)	6.9 (±1.9)	30.2 (±10.9)
105#	19.2 (±2.2)	82.9 (±10.3)	4.0 (±1.9)	17.1 (±10.3)

choices ($p < 0.0001$) and mean number of choices ($p < 0.0001$). No statistically significant difference could be found in the 0 g/kg exposure in terms of mean percentage choices or mean number of choices.

Discussion

After 7 and 14 days exposure only the 75, 90 and 105 g/kg exposure groups differed statistically significantly from the control in terms of mortality (Figure 1). This is an indication of *P. laevis*' tolerance to lead nitrate contamination. The LC_{50} is extremely high when compared to that of other soil invertebrates, such as earthworms (Neuhauser, Loehr, Milligan & Malecki 1985). Terrestrial isopods are known to compartmentalize and detoxify metals in their hepatopancreas (Hopkin & Martin 1982; Hopkin 1990b). This phenomenon may partly explain the tolerance these animals have to the high concentrations of lead nitrate used in this study. The mortalities observed in these tests could be due to the fact that the lead-containing granules in the hepatopancreas become saturated after a certain period, which may cause the excess lead to pass into the blood and interfere with biochemical reactions in other tissues (Jones & Hopkin 1996). Energy is needed for repair mechanisms if a contaminant exceeds toxic levels (Calow 1989). This will reduce the energy available for normal functioning and will increase the probability of death (Donker 1992).

The isopods of the groups exposed to various concentrations of lead nitrate differed significantly from the control after 7 and 14 days exposure in terms of mass loss or gain (Figure 2). Thus, mass changes are a more sensitive measure of the effects of lead nitrate on *P. laevis* than mortality. Results obtained in the present study revealed that *P. laevis* has the ability to distinguish and avoid leaves contaminated with lead nitrate concentrations (15 and 105 g/kg) used in the acute toxicity tests. Apart from compartmentalization, avoidance can be used as a mechanism to resist contaminants, as the only significant route of metal uptake is via the food (Hopkin & Martin 1984). Organisms may take direct energy costs to resist a contaminant by avoidance (Donker 1992), thereby leaving less energy for processes such as growth and reproduction. The isopods in the acute toxicity tests could have lost mass because of energy lost in resisting the lead nitrate by avoidance. Although the solutions were sprayed on as homogeneously as possible, using a mist sprayer, the possibility of uneven application existed. The isopods could have fed selectively on the leaves to some extent.

The concentrations of lead found in the isopods after 14 days exposure to various high concentrations of lead nitrate (Table 1) show that although these animals can distinguish lead nitrate, they still eat the food presented to them. The food consumption rate may have been lowered as found in *P. scaber* by Van Capelleveen *et al.* (1986). The lead body burden of *P. laevis* is much lower than the concentrations to which these isopods were exposed (Table 1). Note that the body burdens are given in mg/kg but exposure concentrations in g/kg. However, the concentrations found are still high when compared to concentrations found in isopods in previous studies (Hopkin & Martin 1982; Hopkin 1990b) and could give a possible explanation for the mortalities and mass losses observed, as lead at those concentrations could have disturbed the normal physiological functioning of the animals. Metals can poison isopods if the capacity of the metal-containing granules in the hepatopancreas is exceeded and no further detoxification can take place (Hopkin 1990a).

The reduction in decomposition rate in lead polluted leaf litter (Hopkin 1994) may be partially explained by the results of this study. Soil invertebrates are directly responsible for

only about 5–10% of the chemical decomposition of leaf litter (Peterson & Luxton 1982). However, they act as 'catalysts' by stimulating the activities of bacteria and fungi which conduct the majority of chemical decomposition (Anderson 1988; Bengtsson, Berden & Rundgren 1988). They do this by fragmenting leaf litter into small particles which are voided as feacal pellets. These pellets provide a more favourable substrate for microbial breakdown (Eisenbeis & Wichard 1987). The decomposition of organic matter in leaf litter provides the nutrients required to keep a forest productive. When metals such as lead are deposited from the air, the soil litter binds it tightly and may accumulate high concentrations. Sites heavily contaminated with lead and other heavy metals have smaller populations of decomposer organisms such as fungi, earthworms, and arthropods (Bengtsson & Rundgren 1984; Beyer & Anderson 1985), thus resulting in the reduction of biological activity that might have implications for decomposition processes, as evidenced by accumulating litter (Freedman & Hutchinson 1980; Strojan 1978). The results of this study showed that the animals avoided leaves contaminated with lead nitrate, implying accumulation of leaf litter and thus a reduction of the decomposition rate. Terrestrial isopod populations may not be reduced by moderate levels of lead contamination because of the high tolerance to lead as manifested in the LC_{50} values obtained for these animals.

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