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Growth Retardation and Elemental Differences in Juvenile *Armadillidium vulgare* Latreille Exposed to Lead Nitrate

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Although it is well established that isopods store heavy metals, especially zinc, cadmium, lead and copper (Williamson and Evans 1972; Brown 1976, 1978; Beeby 1978; Joose *et al.* 1983; Hopkin and Martin 1984; Hopkin *et al.* 1985, 1986), there is relatively little known about the effects of these metals on the isopod. Hopkin and Martin (1984) studied the uptake of zinc, cadmium, lead and copper in juvenile *Oniscus asellus* over a six-week period. Isopods were fed either uncontaminated leaf litter or zinc, cadmium, lead and copper-contaminated leaf litter. Although not discussed, their data indicate the mean dry weights of newly-released juveniles increased 82% over the six-week period when fed uncontaminated leaf litter. However, the juvenile isopods from the same batch that were fed contaminated leaf litter increased only 55% in dry weight over the same time period. Growth retardation has also been reported in terrestrial isopods exposed to sublethal levels of zinc and manganese (Joose *et al.* 1983) and in the freshwater isopod *Asellus* exposed to lead or copper (Brown 1976). However, Hopkin (1990) indicated that there was no effect on growth of adult isopods exposed to a combination of metals over a twenty-week period.

The literature is sparse relating to the effects of non-essential heavy metals on concentrations of essential elements in isopods. Hopkin and Martin (1982) studied the relative concentrations of heavy metals in the hepatopancreas of *Oniscus asellus* from a lead-contaminated site. Although leaf litter concentrations of zinc, copper and cadmium were essentially the same between the uncontaminated and lead-contaminated sites, the hepatopancreas tissue concentrations

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were 1.3 times higher for copper and 6 times higher for zinc in the contaminated isopods than in the uncontaminated. Hopkin *et al.* (1985) indicated that *Ligia oceanica* residing near zinc, cadmium and lead-contaminated sites accumulated more of these elements, as well as copper, than those from an uncontaminated site. The contaminated isopods assimilated insignificant concentrations of lead in their tissues. In a recent study (Tomita *et al.* 1991), adult *A. vulgare* exposed to lead sequestered copper and zinc in the hepatopancreas to levels far above the controls. In the present study, the effect of lead nitrate on *A. vulgare* recently released from the brood pouch was examined.

MATERIALS AND METHODS

Adult pregnant *A. vulgare* were collected from an uncontaminated site in the Bronx, New York, and reared in the laboratory on uncontaminated or lead-contaminated leaf litter. At the start of the experiment, 3 ml of spring water or 3 ml of a lead solution of either 1 mg/L or 10 mg/L lead nitrate (see Beeby 1978) in spring water were added to the leaf litter in each culture dish. The females were reared for a period of four to six weeks before the broods were released. The females were then removed, and the broods in each of the study groups were maintained in their respective assigned groups for a total period of 101 days. Moderate humidity was maintained throughout the duration of the experiment. During the study period, the laboratory temperature varied between 24.5-27.0°C with approximately 12 hours of light daily.

At 101 days, 48 juvenile isopods were randomly selected and their lengths were individually measured (N=14 from the controls, N=17 from each of the lead nitrate groups). The remaining isopods in each study group, excluding those measured for lengths, were used for dry weight measurements and elemental analysis (for copper, zinc, calcium and lead) by flame atomic absorption spectrophotometry (AAS)(N=163: N=13 from the controls, N=20 groups of 5 from the 1 mg/L lead nitrate group and N=10 groups of 5 from the 10 mg/L lead nitrate group). These isopods were transferred to a large dish containing sterile, ultra-pure deionized water and washed five to six times over a period of twenty to thirty minutes, transferring the isopods to clean dishes between washes. The isopods were then transferred to a clean dish and stored at 60°C for a period of four weeks. At that time, control specimens were weighed individually. Due to their low weights, the isopods in the 1 mg/L and 10 mg/L lead nitrate groups were pooled in groups of five, weighed and digested completely for atomic absorption analysis. Specimens were digested in 1 ml of concentrated nitric acid (spectral grade) in open 13 X 100 mm glass tubes for approximately one hour at 120-125°C and brought to a total volume of 2.5 ml with ultra-pure deionized water (Hopkin and Martin 1982; Tomita *et al.* 1991). The concentrations of metals were determined by the flame method using a Perkin-Elmer atomic absorption spectrophotometer. The detection limits were as follows: 0.001 mg/L for copper, 0.0008 mg/L for zinc, 0.001 mg/L for calcium and 0.01 mg/L for lead. The Statistical Package for the Social Sciences (SPSSX) on the Digital VAX mainframe was used for data base management and statistical analysis. MANOVA, 2-way ANOVA, multiple regression and Pearson's Correlation statistical tests were done.

RESULTS AND DISCUSSION

The results of this study reveal growth retardation and elemental changes in juvenile isopods exposed to lead nitrate. Body length of the isopods decreased with increasing concentrations of lead nitrate (Table 1). Multivariate analyses of dry weights (Table 2) and lengths (Table 1) indicate significant effects for lead nitrate dose ($P<.001$) and 2-way ANOVA reveal significant differences between all groups ($P<.0001$). Likewise, there were significant differences in weights and lengths ($P<.0001$) between the control and 1 mg/L lead nitrate group, the control and 10 mg/L lead nitrate group and the 1 mg/L and 10 mg/L lead nitrate groups. Regression of weight and length data indicate significances for lead nitrate dose (Table 3). However, eta (E) values, a measure of association, for weights ($E=.9881$) and lengths ($E=.9286$) versus lead nitrate dose are greater than the corresponding R values ($R=.8021$ and $R=.8842$, respectively). Thus, the nature of the association between the variables lead nitrate dose and weights and lengths appear to be curvilinear. These findings are similar to those of Brown (1976).

There are relatively low concentrations of lead in the treated isopods (Table 2). There are also insignificant negative correlations ($P>.05$) between lead and the other elements. The distributions of lead values in all groups are skewed, with the best measure of central tendency being that of the mode equal to zero. Beeby's (1978) study of *Porcellio scaber* indicated minimal assimilation of lead. Williamson and Evans (1972), however, noted substantial increases in the lead content of

Table 1. Summary of length measurements of juvenile *A. vulgare* exposed to varying concentrations of lead nitrate over a period of 101 days (Mean±SD). 2-way ANOVA significances reported across all groups.

Group	Length(mm)[1]
Control (N=14)	5.46±.38[a]
1 mg/L Lead Nitrate (N=17)	3.33±.50[b]
10 mg/L Lead Nitrate (N-17)	2.49±.31[c]
Significance	P<.0001

[1] Dissimilar alphabets represent 2-way ANOVA significant differences between groups (P<.0001).

Table 2. Summary of AAS results of juvenile *A. vulgare* exposed to varying concentrations of lead nitrate over a period of 101 days (Mean±SD). 2-way ANOVA significances reported across all groups.[1]

Group	Dry Wt µg/g	Copper µg/g	Zinc µg/g	Calcium mg/g	Lead µg/g[2]
Control (N=13)	1.92±.26[a]	469±42[a]	255±68[a]	175±9[a]	32±58[a]
1mg Pb(N=20)[3]	0.49±.20[b]	416±146[a]	296±63[a]	139±24[b]	76±95[a]
10mg Pb (N=10)[3]	0.22±.11[c]	798±252[b]	784±252[b]	168±50[a]	16±49[a]
Significance	P<.0001	P<.001	P<.001	P<.001	P>.05

[1] Dissimilar alphabets represent significant differences between groups (P<.001) by 2-way ANOVA.
[2] Skewed distributions with Mode=0.
[3] N represents the number of groups of five isopods.

isopods from a lead-contaminated site when compared to other invertebrates in the same area. Lead accumulation has also been reported in the freshwater isopod *Asellus* (Brown 1976, 1978; Fraser 1980; Lewis and McIntosh 1986). It is possible that the lead in the soil or leaf litter (Williamson and Evans 1972;

Hopkin and Martin 1982, 1984; Hopkin *et al.* 1985) is in a much more readily available form to these isopods than the lead nitrate added to leaves (Beeby 1978; Tomita *et al.* 1991). Hopkin and Martin (1984) suggested that in feeding experiments such as this the isopods may be reacting to the concentrations of anions rather than to differences in the levels of metals. Anionic interactions and effects on metal absorption were not analyzed in this study.

Multivariate analyses reveal significant effects for lead dose ($P<.001$) and 2-way ANOVA analyses reveal significant differences between all groups for copper, zinc and calcium ($P<.001$)(Table 2). The main effects for the copper and zinc significances appear to stem from the comparisons between the 10 mg/L lead nitrate group and either the control or the 1 mg/L lead nitrate group. The main effect for the calcium significance appears to be from the influence of the 1 mg/L lead nitrate group when compared either to the control or the 10 mg/L lead nitrate group.

The elements remained positively correlated with one another (Table 3). Regression analyses of the elements versus lead nitrate dose reveal significances for copper and zinc (Table 3). In the case of copper (Table 2), there is a mean decrease with an increased variability in the 1 mg/L lead nitrate group when compared to the control group. The copper concentration in the 10 mg/L lead nitrate group is approximately 1.7 times that of the control group. The nature of the relationship between lead nitrate dose and copper concentration appears to be curvilinear ($E=.7375$, $R=.5167$) as are weights and lengths. The curvilinear relationship is also true to a lesser extent for zinc ($E=.8594$, $R=.7273$). These findings are consistent with those of adult *A. vulgare* exposed to lead nitrate (Tomita *et al.* 1991).

Our previous study on the effects of lead nitrate on adult *A. vulgare* (Tomita *et al.* 1990,1991) showed an increase in copper and zinc levels in the hepatopancreas of those isopods exposed to lead. The increase in copper and zinc levels could be due to an increase in the absorption of these metals from the environment or to a shift in these metals from other areas of the isopod to the hepatopancreas. Since whole isopods were analyzed in this study, and copper and zinc concentrations are elevated in those isopods exposed to lead nitrate, the source of the copper and zinc must be exogenous and not simply due to a shift of metals from one region of the animal to another. Increased bioaccumulation of copper and zinc in those isopods exposed to lead raises interesting questions regarding the influence of lead on absorption and regulation of essential elements.

Table 3. Summary of correlations and regressions of weights, lengths and AAS results for copper, zinc and calcium from juvenile *A.vulgare* exposed to varying concentrations of lead nitrate over a period of 101 days.

Correlations Variables	r	Significance
Wt vs Copper	-.2798	.038
Wt vs Zinc	-.4859	.001
Wt vs Calcium	.2958	.030
Copper vs Zinc	.7545	<.001
Copper vs Calcium	.5498	<.001
Calcium vs Zinc	.3360	<.016
Regressions Variables	R^2	
Weight	.6435	<.0001
Length	.7818	<.0001
Copper	.2670	<.0005
Zinc	.5290	<.0001

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