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Slow Accumulation of Lead from Contaminated Food Sources by the Freshwater Gastropods, *Physa integra* and *Campeloma decisum*

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Abstract. Rates of lead accumulation from contaminated food sources were examined for the freshwater gastropods, *Physa integra* and *Campeloma decisum*. Lead was slowly accumulated from *aufwuchs* (0.52–0.87 μg Pb/g dry wt of tissue/day) by the grazing snail, *P. integra* while no statistically significant lead accumulation from sediments was noted in the deposit/suspension feeder, *C. decisum*. *Aufwuchs*-associated iron and manganese did not affect the bioavailability of lead.

The relative significance of dietary and water sources of lead to aquatic biota has been the focus of much research and speculation, yet remains generally unresolved. Waterborne lead is readily absorbed by rainbow trout (*Salmo gairdneri*) while dietary lead is accumulated only slowly (Hodson *et al.* 1978). In contrast, the blue mussel, *Mytilus edulis*, assimilates 24% of the lead in ingested food and 29% in the surrounding seawater (Schulz-Baldes 1974). Furthermore, Bryan and Hummerstone (1978) indicated that the correlation between lead levels in the deposit-feeding bivalve, *Scrobicularia plana*, and surrounding sediments resulted from the assimilation of sediment-bound lead. Changes in lead concentrations in the grazing gastropod, *Physa integra*, were correlated with changes in dissolved lead concentrations in the water column but not changes in lead concentrations of *aufwuchs* in a reservoir characterized by elevated lead levels (Newman and McIntosh 1982). In this same survey, lead concentrations of the infaunal, deposit/suspension feeding gastropod, *Campeloma decisum*, were not correlated with concentration in any potential sediment or water source. Individuals of these two species inhabiting a lead impacted system displayed widely differing soft tissue lead concentrations. *C. decisum* had lowest tissue lead concentrations despite its close association with the contaminated sediments.

Clearly, tissue lead concentrations are determined by the ecological and physiological characteristics of a species, as well as the concentrations of the various forms of lead in food and water sources (Coughtrey and Martin 1977). Therefore, when species specific data are lacking, it is difficult to ascertain the relative significance of potential lead sources. In past work, the authors have described several factors contributing to the widely differing soft tissue lead concentrations of two common and widespread freshwater gastropods, *Physa integra* and *Campeloma decisum*. The research described herein examines the accumulation of lead from two highly contaminated food sources in order to further define the factors contributing to the observed differences in soft tissue lead concentrations in these potential biomonitoring species.

Materials and Methods

Collection Sites

Snails and *aufwuchs* were collected from two contiguous reservoirs (Weston's Mill Pond and Farrington Lake) on Lawrence Brook (Middlesex County, NJ). Lead contamination at these sites is described in detail elsewhere (Newman and McIntosh 1982). The average hardness, total alkalinity, and pH values in these reservoirs were approximately 34 mg/L as $CaCO_3$, 15 mg/L as $CaCO_3$, and 6.85, respectively.

General

A continuous-flow system previously described (Newman and McIntosh 1983) was used to provide a constant flow (7 L/day/

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Table 1. Chemical parameters of the waters during the two accumulation experiments (Mean ± 1 SD)

Experiment	Treatment	Dissolved lead	Temperature (C)	pH	Dissolved oxygen (mg O_2/L)	Hardness (mg/L as $CaCO_3$)	Total alkalinity (mg/L as $CaCO_3$)
	Pb/Fe/Mn in μg/g						
Accumulation	0.06/170/41	0.2 ± 0.2	18.7 ± 0.5	6.51 ± 0.17	7.2 ± 0.6	31 ± 4	15 ± 2
from *aufwuchs*	114/17,000/40,000	0.2 ± 0.1	18.9 ± 0.2	6.53 ± 0.19	7.6 ± 0.6	29 ± 4	14 ± 1
by *P. integra*	126/8,500/98,000	0.3 ± 0.2	18.7 ± 0.5	6.57 ± 0.13	7.7 ± 0.5	31 ± 3	14 ± 1
	130/8,200/97,000	0.2 ± 0.2	18.5 ± 0.5	6.58 ± 0.15	7.7 ± 0.7	29 ± 6	14 ± 1
	μg Pb/g dry wt						
Accumulation	<3.6	0.4 ± 0.1	18.2 ± 0.5	6.66 ± 0.37	7.3 ± 0.8	29 ± 1	15 ± 1
from sediments	252	0.1 ± 0.1	18.6 ± 0.6	6.31 ± 0.23	7.0 ± 0.8	28 ± 3	11 ± 3
	337	0.2 ± 0.1	18.8 ± 0.9	6.43 ± 0.39	6.6 ± 1.2	26 ± 2	11 ± 2
	647	0.3 ± 0.3	18.7 ± 0.7	6.43 ± 0.34	6.7 ± 0.7	28 ± 1	12 ± 2

tank) of artificial freshwater through 3.5 L plastic tanks. The artificial freshwater was made by reconstituting distilled-deionized water with appropriate amounts of $MgSO_4$, $NaHCO_3$, $CaSO_4 \cdot 2H_2O$ and KCl. A light/dark cycle of 14L/10D and test water temperature of approximately 18°C were maintained. The hardness, total alkalinity, and pH were adjusted to the range of the sampling site waters by varying the amounts of the four component salts (EPA 1978). Specific characteristics of the test water for each experiment are given in Table 1.

Lead Accumulation from Aufwuchs by Physa

Previous work (Newman *et al.* 1983) suggested that iron and manganese concentrations in *aufwuchs* (procedurally defined as material accumulating on submerged surfaces) may influence the lead accumulation by grazing gastropods. To test this hypothesis, slides were placed at various locations within the Lawrence Brook sampling area in an effort to acquire materials with different relative concentrations of lead, iron, and manganese.

Snails collected from Weston's Mill Pond in April 1981 were maintained in artificial freshwater and fed pulverized Purina Mouse Chow® until the beginning of the experiment. This period of six weeks was sufficient for clearance of a large portion of the lead accumulated by the snails (Newman and McIntosh 1983). One hundred specimens were randomly placed into each of 12 tanks in the continuous-flow system. Snails were not fed for the next two days. During the experiment, snails in tanks 1 through 3 were fed Purina Mouse Chow® (metal concentrations in μg/g dry wt = Pb: 0.06; Fe: 170; Mn: 41). Snails in tanks 4 through 6 grazed upon material accumulated on slides which had been suspended in the waters of Farrington Lake for two months prior to the beginning of the experiment (metal concentrations in μg/g dry wt = Pb: 114; Fe: 17,000; Mn: 40,000). Snails in tanks 7 through 9 fed upon material from slides similarly cultured in an area of Weston's Mill Pond approximately 0.2 km upstream of Ryders Lane (metal concentrations in μg/g dry wt = Pb: 126; Fe: 8,500; Mn: 98,000). The snails in the remaining tanks (10 through 12) grazed upon material accumulated on slides which had been suspended in Weston's Mill Pond immediately adjacent to the heavily traveled Ryders Lane (metal concentrations in μg/g dry wt = Pb: 130; Fe: 8,200; Mn: 97,000). In this manner, *P. integra* were exposed to food containing low concentrations of lead (Mouse Chow®) or *aufwuchs* containing approximately 125 μg Pb/g dry wt but differing iron and manganese concentrations.

Three slides were added to each tank initially. Two of the slides had one side scraped clean of material for metal analyses before being added to each tank. The scraped materials from six slides (two from each of three tanks) used in each treatment were pooled, dried, and analyzed for lead, iron, and manganese concentrations. A new set of three slides was added to each of the tanks every third day. To maintain six slides in each tank continuously, the three slides which had been in the tank longest were removed at each feeding. The percentage of all snails feeding in each tank was noted daily. On days during which slides were added to the tanks, snails were allowed five hr to find the fresh material before percentage feeding was determined. After each feeding, feces and materials accumulating in the tanks were removed with a siphon.

Ten snails from each tank were sampled after 0(4hr), 3, 7, 11, 15, 19, 23 and 25 days. They were allowed 4 hr in fresh artificial freshwater to clear their guts and then frozen. Later, specimens were removed from their shells, pooled, and analyzed for lead concentrations.

Lead Accumulation from Sediments by Campeloma

Snails were collected from the Farrington Lake site one week (March 1981) before being placed into the continuous-flow system. In tanks 1 through 3, clean sand (lead concentration less than 3.6 μg Pb/g dry wt) was added to provide a substrate into which snails could burrow. In the remaining nine tanks, Weston's Mill Pond sediments were added which contained different concentrations of lead. In tanks 4 through 6, the sediments contained 252 ± 28 μg Pb/g dry wt. Sediments in tanks 7 through 9 contained 337 ± 9 μg Pb/g dry wt, while the remaining tanks (10 through 12) contained sediments with 647 ± 46 μg Pb/g dry wt. Eighteen snails were placed into each of the twelve tanks. Each tank received a continuous flow of artificial freshwater with low lead concentrations (Table 1). Only snails in tanks 1 through 3 were fed Purina Mouse Chow® (lead concentration = 0.09 ± 0.01 μg Pb/g dry wt) as the sediments provided a source of food for these deposit/suspension feeders in tanks 4 through 12. Snails were sampled after 0(4hr), 3, 6, 9, 14, 21, and 30 days. At each sampling, two snails were removed from each tank and allowed four hr to clear their guts before being frozen. Snails were thawed, measured for shell length, removed from their shells, and analyzed for lead concentrations.

Table 2. Metal concentrations in food and biological parameters measured during the experiment designed to assess lead accumulation from *aufwuchs* by *Physa*

Tanks	Concentration in food (μg/g dry wt)			Shell length (mm)	Snail observed feeding on the slides %
	Pb	Mn	Fe		
1–3	0.06 ± 0.01	41 ± 4	170 ± 7	6.3 ± 0.8	—
4–6	114 ± 28	40,000 ± 5,000	17,000 ± 5,000	6.3 ± 1.0	19 ± 11
7–9	126 ± 27	98,000 ± 20,000	8,500 ± 1,500	6.2 ± 0.9	20 ± 13
10–12	130 ± 17	97,000 ± 20,000	8,200 ± 1,800	6.2 ± 0.9	19 ± 13

All values are mean values ± SD

Metal Analyses

Methods for analysis of tissue, sediment and water have been described in detail elsewhere (Newman and McIntosh 1982). Briefly, tissue and sediment samples were digested at 55°C for six hr in concentrated nitric acid. Sediment samples were analyzed for lead, iron, and manganese using a Perkin-Elmer Model 603 atomic absorption spectrophotometer. Tissue digests and dissolved lead in water (passing through a 0.45 μm filter) were analyzed by a Perkin-Elmer 503 atomic absorption spectrophotometer equipped with an HGA 2100 flameless unit. A deuterium arc lamp was used for background correction, while standard additions were used to check for matrix effects.

Results

Lead Accumulation by Physa

Metal concentrations in the food, as well as snail sizes and percentage of snails observed feeding, are given in Table 2. Concentrations of all three metals (lead, manganese, and iron) were low in Mouse Chow®. Lead concentrations in all of the *aufwuchs* samples were approximately 125 μg Pb/g dry wt, although the iron and manganese concentrations were different. About 19 to 20% of the snails were observed feeding in all three *aufwuchs* treatments. Figure 1 shows the changes in *P. integra* lead concentrations over time in all four treatments. Snails feeding on Mouse Chow® had low (approximately 17 μg Pb/g dry wt) and invariant lead concentrations for the duration of the experiment (Figure 1d). The slope of the line of best fit for these data (the rate of lead accumulation) was not significantly different from zero ($\alpha = 0.05$; $r = -0.03$; $t = -0.16$; $n = 26$). In contrast, snails feeding on the *aufwuchs* displayed a linear increase in body lead concentrations. Snails exposed to material containing 114 μg Pb/g dry wt, 17,000 μg Fe/g dry wt and 40,000 μg Mn/g dry wt accumulated lead at a rate of 0.87 μg Pb/g dry wt of tissue/day (Figure 1c). The slope (rate of accumulation) was significantly different from zero at $\alpha = 0.05$ ($r = 0.70$; $t = 4.86$; $n = 27$). Snails exposed to material containing approximately the same lead concentrations but higher manganese and lower iron concentrations accumulated lead at a similar rate (Figures 1a & 1b). Lead accumulation rates in snails exposed to these treatments ranged from 0.52 to 0.84 μg Pb/g dry wt of tissue/day. Both slopes (rates of accumulation) were significantly different from zero at $\alpha = 0.05$ (Figure 1a: $r = 0.72$; $t = 5.22$; $n = 27$; Figure 1b: $r = 0.75$; $t = 5.65$; $n = 27$).

Lead Accumulation from Sediments by Campeloma

The shell length (mm) measured for snails sampled from the <3.6, 252, 337 and 647 μg Pb/g dry wt treatments were 21.2 ± 3.9, 20.9 ± 4.2, 20.9 ± 2.8 and 19.8 ± 2.2, respectively. Approximately 80% of the snails were gravid. Neonates were found in the sediments from the first week of the experiment onward. Figure 2 shows the changes in lead concentrations for the snails during the 30 days of exposure. None of the rates of accumulation (slopes) were significantly different from zero ($\alpha = 0.05$) in any of the four treatments. A slow clearance of lead was indicated in the treatment receiving Mouse Chow®. As the *C. decisum* were collected from an area with 210 μg Pb/g dry wt in the sediments, it was not unexpected that snails exposed to sediments with lead concentrations of 252 and, even 337 μg Pb/g dry wt would show little uptake during the 30 days of exposure. However, snails exposed to sediments with 647 μg Pb/g dry wt also showed no significant increase in tissue lead concentrations.

Tissue lead concentrations, as well as body lead concentrations of late-stage embryos, neonates and adult snails, are shown in Table 3. Data for *C. decisum* exposed to <3.6 μg Pb/g dry wt indicated a decrease in lead in several tissues, especially the

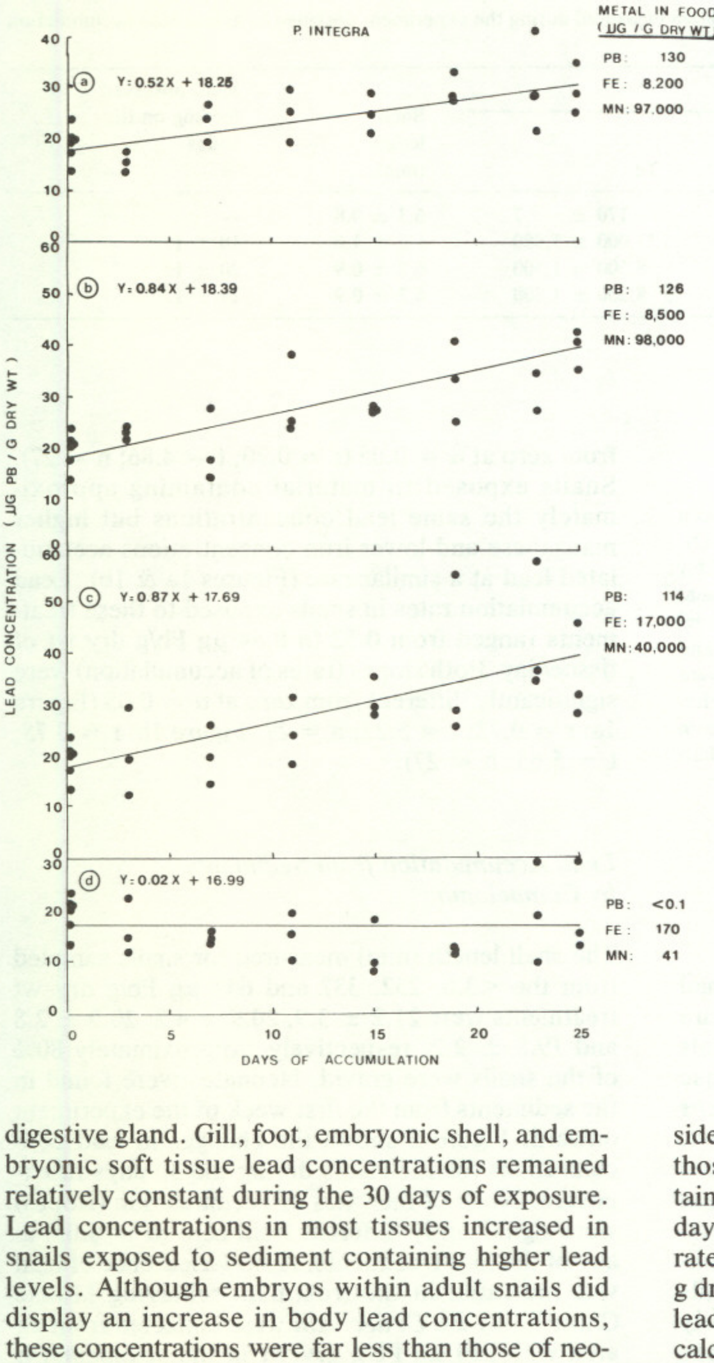

Fig. 1. Lead accumulation by *P. integra* fed Mouse Chow® which contained low concentrations of lead (d) or *aufwuchs* containing approximately 125 µg Pb/g dry wt and varying amounts of iron and manganese (a, b and c)

digestive gland. Gill, foot, embryonic shell, and embryonic soft tissue lead concentrations remained relatively constant during the 30 days of exposure. Lead concentrations in most tissues increased in snails exposed to sediment containing higher lead levels. Although embryos within adult snails did display an increase in body lead concentrations, these concentrations were far less than those of neonates exposed directly to the sediments. Neonates displayed lead concentrations 5 to 16 times higher than those in embryos. In contrast, adult snails had slight or no increase in total body lead concentrations.

Discussion

Rates of lead accumulation from *aufwuchs* containing approximately 125 µg Pb/g dry wt were considerably lower (0.52–0.87 µg Pb/g dry wt/day) than those reported for *P. integra* from plant tissue containing 28 ± 3 µg Pb/g dry wt (3.65 µg Pb/g dry wt/day) (Newman 1981) and slightly greater than the rate from water containing 2 µg Pb/L (0.34 µg Pb/g dry wt/day)[2]. The relative availability of these three lead sources to *P. integra* can be approximated by calculating the rate of change of the concentration factor (concentration in the organism/concentration in the source). When the above data are transformed in this manner, the following rates are derived ((µg Pb per g dry wt/µg Pb per g)/day): water = 170.0; plant tissue = 0.13; and *aufwuchs* = 0.006. Lead dissolved in the water was accumulated

[2] Extrapolated from Figure 7 (Spehar *et al.* 1978). This value should be considered as a crude estimator only, as accumulation was assumed to be linear during the 28 days of lead exposure. Extrapolation to 2 µg Pb/L was also necessary.

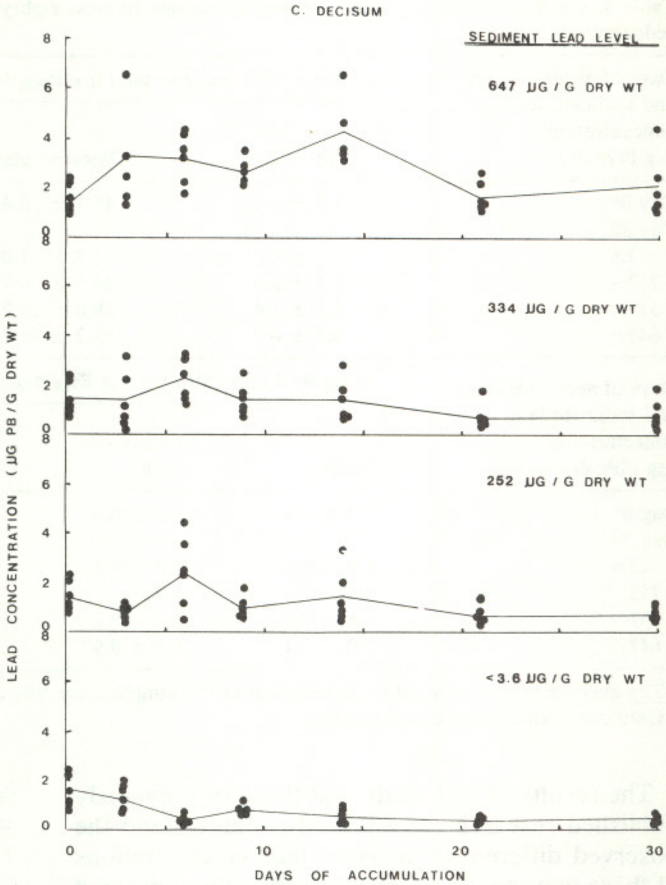

Fig. 2. Lead accumulation by *C. decisum* exposed to different lead concentrations in sediment

approximately 28,000 times more readily than lead in *aufwuchs,* while lead in plant tissue was accumulated 20 times more readily than lead associated with *aufwuchs*.

Differences in iron and manganese concentrations in *aufwuchs* did not affect lead accumulation rates in this study. There could be two explanations for this observation: 1) the range of concentrations was not sufficiently wide to produce a detectable difference in lead accumulation; 2) the original hypothesis that iron and manganese (hydrous oxides) may interfere with lead accumulation was incorrect. In the work of Luoma and Bryan (1978) in which the influence of iron on lead accumulation in a deposit-feeding bivalve was demonstrated, the ratio of Pb:Fe in 1 N HCl sediment extracts varied by two orders of magnitude. The ratio of µg Pb: mg Fe in the present study ranged from 7 to 16. Perhaps the iron and manganese did interfere with lead accumulation in the present study, but this interference was not detectable. Further work is necessary in this area to substantiate these findings.

Adult *C. decisum* accumulated lead from contaminated sediments very slowly. This characteristic likely contributes to the snail's ability to maintain relatively low lead concentrations when exposed to lead contaminated sediments in the field (Newman and McIntosh 1982). At the end of the experiment, highest lead concentrations were found in the digestive gland and gestatory sac while lowest concentrations were found in the foot tissue. When adults, late-stage embryos, and neonates were exposed to the same level of contamination, neonates accumulated more lead than either adults or embryos (exposed indirectly to the sediment-bound lead via the adult). Assuming the late-stage embryos and neonates possess similar metabolic rates, the following explanation is advanced. Using the approach of Boyden (1974, 1977), Newman and McIntosh (1983) found that the relationship between body size and lead content of *C. decisum* was determined either by: 1) a mechanism linked directly to the snail's metabolic rate, or 2) the surface-to-volume ratio of the snail. If embryos possess metabolic rates similar to neonates and were exposed to the sediment-bound lead through the surface of the parent, then differences seen in the body lead concentrations of neonates and embryos were likely a result of the surface of exposure to volume of tissue ratio, not a mechanism linked directly to the metabolic rate. Obviously, further investigations are necessary to confirm or negate this theory.

Table 3. Comparison of lead accumulation in various tissues, embryos, neonates and adult *C. decisum* exposed to contaminated sediments

Days of accumulation and sediment lead concentration (μg Pb/g dry wt)	Mean lead concentration (μg Pb/g dry wt)			
	Gill	Digestive gland	Gestatory sac	Mantle
Day 0[a]	1.7 ± 0.9	15.3 ± 5.4	5.8 ± 4.0	4.3 ± 2.7
Day 30				
<3.6	2.1 ± 0.6	2.9 ± 1.0	1.6 ± 0.3	1.8 ± 0.1
252	7.4 ± 3.3	24.5 ± 10.7	26.9 ± 10.6	4.8 ± 2.8
337	5.1 ± 1.6	28.0 ± 13.2	20.7 ± 4.5	2.3 ± 0.1
647	6.7 ± 0.8	135.2 ± 41.7	88.1 ± 58.5	15.8 ± 4.3

Days of accumulation and sediment lead concentration (μg Pb/g dry wt)	Mean lead concentration (μg Pb/g dry wt)				
			Whole body (soft tissues)		
	Foot	Embryonic shell	Embryo	Neonate	Adult
Day 0[a]	1.2 ± 0.5	1.0 ± 0.4	0.6 ± 0.0	—	1.5 ± 0.6
Day 30					
<3.6	1.0 ± 0.0	1.0 ± 0.5	0.3 ± 0.1	1.6 ± 0.1	0.4 ± 0.2
252	2.3 ± 0.8	1.9[b]	4.7[b]	25.6 ± 11.3	0.8 ± 0.3
337	1.6 ± 0.1	2.3 ± 0.2	3.8 ± 5.0	37.5 ± 13.4	1.0 ± 0.6
647	5.0 ± 3.1	1.5 ± 0.9	5.4 ± 0.8	85.9 ± 10.9	2.1 ± 1.3

[a] The average lead concentration in sediment at the sampling site was 210 μg Pb/g dry wt
[b] Only one pooled sample was analyzed

The results of this study and those of previously published research can be used to understand the observed differences in tissue lead concentrations of these two species when exposed to the same lead contaminated environment (Newman and McIntosh 1982). *P. integra* accumulates lead readily from water (Spehar *et al.* 1978), plant tissue (Newman 1981) and *aufwuchs* (present study). Despite earlier indications (Newman and McIntosh 1982; Newman *et al.* 1983), the large amount of abiotically-bound lead present in the procedurally-defined *aufwuchs* does not appear to exclude this material as a significant source of lead to this species (present study). Although a large portion of the lead accumulated by *P. integra* can be rapidly eliminated, a significant pool of strongly-bound lead is only very slowly eliminated from the tissues of this snail (Newman and McIntosh 1983).

In contrast, *C. decisum* accumulates lead very slowly from the contaminated sediments (Newman and McIntosh 1983; present study). Although, accumulated lead in the tissues of this species is very slowly eliminated, the pool of strongly-bound lead in *C. decisum* never reaches the levels noted for *P. integra* (Newman and McIntosh 1983). *Campeloma decisum* appears to be able to control the amount of lead accumulating in its tissues. The surface-to-volume ratio of this large snail may contribute to this ability (Newman and McIntosh 1983; present study).

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