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Snail (*Helix aspersa*) Exposure History and Possible Adaptation to Lead as Reflected in Shell Composition

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Abstract. Lead sequestration in shell was examined for English and Welsh populations of the common garden snail (*Helix aspersa*) with different Pb exposure histories. Isotopic Pb ratios provided signatures for Pb source and a means of implying duration of population exposure from decades to millennia. Total Pb concentrations were used to quantify the intensity of exposure experienced by the populations. Snails from populations with long histories of exposure (millennia) to high Pb levels had proportionately more Pb in their shell than soft tissue compared with snails from other surveyed populations. These observations suggest that Pb sequestration in shell has been enhanced in *H. aspersa* populations with long and intense exposure to Pb.

Organisms use a wide range of mechanisms to lessen the effects of toxic metals in their environment. One mechanism, sequestration of toxic metals in relatively inert tissues, has been demonstrated for several invertebrates from contaminated habitats (Beeby 1991). This mechanism can be amenable to selection and, under certain conditions, leads to enhanced tolerance for the associated population. Some populations of *Orchesella cincta* (Collembola) have adapted to high Cd contamination by increasing metal incorporation into the hind-gut lining, which is shed at each molt (Van Straalen *et al.* 1986; Posthuma 1990). Freshwater crustaceans use the carapace to isolate Cd and Pb (Wright 1980). Enhancement of this ability increased tolerance to Pb in some populations of *Asellus meridianus* (Brown 1977).

Slugs, snails and earthworms all tend to have higher levels of Ca in their soft tissues from Pb contaminated sites (Ireland 1979a, 1979b; Beeby and Richmond 1988), suggesting that modified Ca metabolism may be linked to tolerance. Beeby (1991) identified metal sequestration in intracellular granules or shell by modification of essential element metabolism in a population of the common garden snail (*Helix aspersa* Muller) from an urban environment with high ambient Pb. This population of snails displayed Ca metabolism distinct from that of a rural population (Beeby and Richmond 1987, 1988). Further experiments on Pb assimilation with a third population suggested that this capacity may, in part, be determined by a genetic change in the urban population (Richmond and Beeby 1992). A laboratory study of Pb uptake and elimination indicated that snails from the putatively adapted population incorporated relatively more Pb into shell and at a faster rate than snails from the rural, less contaminated site (Beeby and Richmond, 1989, 1991).

Was the enhanced incorporation of Pb into shell by *H. aspersa* a general phenomenon or one peculiar to a single contaminated study site? A survey of *H. aspersa* populations with differing Pb exposure histories could provide a first step in answering this question. Such a survey could document the relative amounts of Pb in soft tissues and shell as an indicator of altered sequestration of this toxic metal. These data then could be interpreted based on the intensity and duration of Pb exposure for each population.

Intensity of exposure could be reflected in Pb concentrations in soft tissue and shell, but estimation of the exposure *duration* is more difficult. Fortuitously, the possibility exists in certain places, such as England and Wales, for use of isotopic signature to indicate the proportion of a metal derived from anthropogenic (automotive) and native sources. The Pb from automotive sources represents recent exposure (decades), while Pb from native sources, including local mines and smelters, reflects long periods of exposure (centuries to two millennia). This work describes the relative intensity and duration of Pb exposure to *H. aspersa* populations using Pb isotopic data, and provides preliminary evidence of population response to Pb exposure as reflected in shell composition.

Materials and Methods

Sample Collection

Thirty-three populations of *H. aspersa* were sampled in England and North Wales during the summer of 1990 (Figure 1 and Table 1). Sites were chosen that were likely to have either major sources of Pb or

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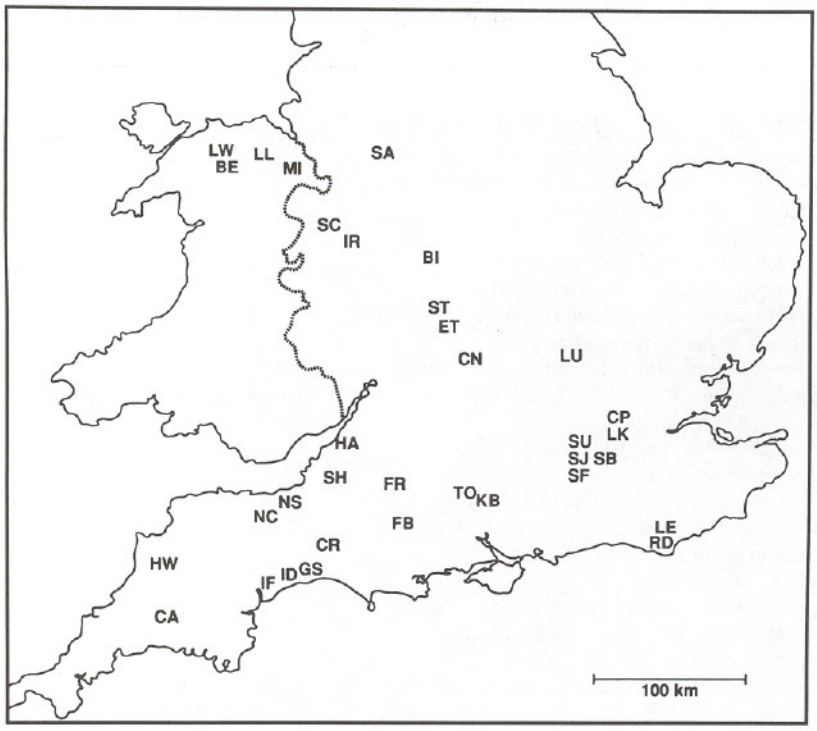

Fig. 1. Locations of the 33 sample sites in England and North Wales. The car park site originally studied by Beeby and Richmond (1987, 1988) is denoted by CP

insignificant metal contamination. The sites were further chosen from areas with a long history of metal working or areas where non-native Pb sources may dominate. The non-native Pb originated primarily from automotive sources. The survey included sites with high mineral Pb and sites where native Pb had been exploited, in some cases since the Roman occupation of the region.

Snails were collected for metal analyses, placed in clean plastic containers and allowed 24 h to clear their gut. They were washed in tap water and fed for 48 h on notionally Pb-free lettuce before being washed again and frozen. Only adult snails (e.g., those possessing a lipped shell) were used for Pb analyses. [The presence of a lip implies an individual older than 4 years for most field populations in the United Kingdom (Comfort 1957, Beeby and Eaves 1983).] Where sufficient numbers were collected (23 sites), five adults were retained by the South Bank Laboratory (SBL) for Pb analyses. Otherwise, all individuals from a site were shipped to the Savannah River Ecology Laboratory (SREL) for Pb analyses.

Shell Analyses

Cost restricted shell isotope analyses to one or two shells per site. Two shells were combined and digested together for total Pb and isotopic analyses for sites initially thought to have low Pb concentrations (ID, KB, LK, RD, SC, SF). All shells were lipped except one of the pooled shells from KB. For the LW and NC sites, six shells were digested and analyzed individually to estimate intershell variability.

Shells processed, washed with deionized water, and sealed in plastic bags at the SREL Class 100 clean room were sent to Chempet Research Corporation for digestion and analysis. Digestions were done in teflonware using hot Ultrex® nitric acid. Lead was separated by standard ion chromatography using a bromide medium followed by a cleanup stage using a chloride medium. The Pb spectra from thermal ionization mass spectrometry (NBS Model 1290 TIMS) were calibrated to either ^{204}Pb or ^{206}Pb. Isotopic fractionation was corrected by 0.05%/mass unit based on analysis of a NBS SRM 981 Pb isotopic standard.

Analytical precision of total Pb replicates was 5% (coefficient of variation or CV) as evidenced by standard material analyses (NIST SRM 277, 1646, and 1633a). Intershell precisions for $^{206}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, and total Pb respectively were 0.9%, <0.2%, and 37% for site LW and 1.6%, 0.3%, and 75% for site NC.

Soft Tissue Analyses

Details of soft tissue analyses differed between SBL and SREL. At SBL, snails were analyzed by flame atomic absorption spectrophotometry (FAA) (Varian 1275 with deuterium lamp background correction). Soft tissue was divided into hepatopancreas and "remainder." The anterior lobe of the hepatopancreas was analyzed, but the posterior lobe was retained for future study of granules. Weights for the two lobes were assumed to be identical during calculations of total soft tissue Pb. Tissues were dried at 80°C for 18 h, digested in boiling Analar® grade nitric acid, and filtered (Whatman 541) before analysis. At SREL, thawed snails were removed from their shells in a Class 100 clean room, rinsed with deionized water, and a very small piece of tissue was taken for electrophoretic analyses. After freeze drying (24 h), the entire mass of soft tissue was digested for 4 h in hot (80°C), Teflon-distilled nitric acid (Newman and Mitz 1988) and analyzed by FAA (Hitachi 180-80 with polarized Zeeman background correction).

National Research Council Canada TORT-1 standard material was used to judge analytical efficacy. Ten percent of all digested samples were TORT-1 materials at SREL and four TORT-1 aliquots were processed during each digestion session (of up to 25 samples) at SBL. The SBL recoveries (consistently 70% for Pb and other metals not reported here) necessitated correction for analyte loss. This loss was traced to the filtration step. Mathematical corrections for the removal of the posterior lobe of the hepatopancreas were also done. The SREL recoveries for Pb as evidenced by TORT-1 results ($n = 5$, mean = 96%) and the minimal amount of tissue removed for electrophoretic analyses suggested that no calculations were required to cor-

Table 1. Location and description of the sample sites. Asterisks indicate sites for which Pb analyses were performed at the Savannah River Ecology Laboratory

Site code	(Ordnance survey map reference)	Site description with pertinent roads noted
BE*	Betws-y-Coed (SH 2799 3557)	Parking lot near a major road (A5) in a Pb-rich area
BI*	Gravelly Hill (SP 4098 2902)	Major highway interchange in Birmingham (M6/A4031)
CA	Callington (SX 3600 6900)	Garden center 40 m from a minor road (A3070)
CN*	Chipping Norton (CP 4315 2273)	Driveway, 10–60 m from minor road
CR	Crewkerne (ST 3445 1095)	Backyard garden 150 m from busy road (A30)
ET	Ettington (SP 4273 2485)	Abandoned garden 60 m from busy road (A422)
FB	Shaftesbury (ST 3863 1233)	Field adjacent to parking lot at the town center
FR	Frome (ST 3816 1456)	Garden center, 2–10 m from busy road (A39)
GS	Lyme Regis (SY 3336 0925)	Backyard garden near a parking lot, 40 m from minor road (A3070)
HA	Hallen (ST 3553 1803)	Backyard garden within 500 m of M5, and 3 km downwind of Avonmouth smelter, area with long history of Pb working
HW	Holsworthy (SS 3300 0350)	Garden center, 20–40 m from busy road (A3072)
ID*	Sidmouth (SY 1350 8980)	40 m from a busy road (A3052)
IF	Sidford (SY 3135 0898)	Garden center, 60 m from a busy road (A3052)
IR	Ironbridge (ST 3674 3033)	Garden, 50 m from busy road
KB*	Stockbridge (SU 4357 1351)	Backyard garden, 40 m from busy road (A30)
LE	Lewes (TQ 5492 1110)	Access road in semirural area
LK	Kennington (TQ 5319 1788)	10 m from major road (A3) in South London
LL	Llanrhaedr (SJ 3486 3111)	Garden in small village adjacent to bypass (A525)
LU	Luton (TL 5054 2226)	20 m of major expressway (M1)
LW	Llanwrst (SH 2798 3616)	Abandoned parking lot in town center, in area with long history of Pb working
MI	Minera (SJ 3274 3518)	Field edge adjacent to minor road in village in area of Pb working; old Cd, Pb mines
NC	Nettlecombe (ST 3056 1379)	Walled garden in 16th century manor, 30 m from minor access road
NS*	Nether Stowey (ST 3185 1399)	Garden center, 30–70 m from busy road (A39)
RD	Rottingdean (TQ 5368 1024)	Garden, 100 m from rural village
SA*	Sandbach (SJ 3759 3608)	Backyard garden in small town, 100 m from road
SB*	Surbiton (TQ 5186 1666)	Backyard in London suburb, 40 m from road
SC*	Shrewsbury (SJ 3486 3111)	Storage area in central town cemetery, 40 m from busy road (A5), old Pb mine in area
SF	Surbiton (TQ 5186 1666)	As SB, except front yard, 3 m from busy residential road
SH	Shipham (ST 3444 1575)	Roadside wall in village in area of Cd, Zn, Pb ores, mined since Roman occupation
SJ*	Surbiton (TQ 5186 1666)	Backyard garden adjacent to SB
ST	Straford (SP 4207 2548)	Lumber yard, 3 m from busy urban road (A34)
SU	Surbiton (TQ 5186 1666)	Backyard garden, adjacent to SJ, behind SF
TO	Stockbridge (SU 4357 2273)	Abandoned garage area, 150 m from KB, 30–50 m from busy road (A30)

rect for recovery or tissue removal. Procedural blanks for both laboratories confirmed minimal contamination during the analytical process.

Isotopic Signatures of Pb Sources

The most extensive survey of isotopic Pb in British rocks was compiled by Moorbath (1962) and updated by Fletcher *et al.* (1993). Galena samples from England and Wales had $^{206}Pb/^{207}Pb$ ratios in the general range of 1.17–1.19. The $^{206}Pb/^{204}Pb$ ratios were approximately 18.01 to 18.48 (Fletcher *et al.* 1993). Most of the ore imported into Europe is derived from Precambrian sources in Canada and Australia, and those sources have isotopic ratios that contrast with native deposits (Kersten *et al.* 1992). Lead used in British petroleum additives is derived from both Canada (Cominco in Toronto) and the Broken Hill Mine in Australia (Associated Octel, pers. comm.). The $^{206}Pb/^{207}Pb$ and $^{206}Pb/^{204}Pb$ ratios of most Canadian sources fall within the range of 1.07–1.16 and 15.6–17.6, respectively. However, a number of post-Precambrian ores have $^{206}Pb/^{204}Pb$ ratios up to 19.37. Reflecting their Precambrian origins, ores from Broken Hill and Mount Isa in Australia have $^{206}Pb/^{207}Pb$ and $^{206}Pb/^{204}Pb$ ratios of approximately 1.04 and 16.15, respectively.

Although the proportions of Canadian and Australian ores which go to produce the British additive are unknown (Associated Octel, pers. comm.), the associated Pb is generally less radiogenic than that manufactured in the United States (Mukai *et al.* 1993) and has a higher proportion of the non-radiogenic ^{204}Pb. Consequently, the $^{206}Pb/^{204}Pb$ ratio is one isotopic character useful for differentiating native Pb from the less radiogenic ores used in petroleum additive production. Native sources were assumed to dominate if the $^{206}Pb/^{204}Pb$ ratio exceeded approximately 18.00. The ^{207}Pb and ^{206}Pb were also used to differentiate between native and non-native sources of Pb based on the two decay series, $^{238}U \rightarrow {}^{206}Pb$ and $^{235}U \rightarrow {}^{207}Pb$. Where native sources dominate, the $^{206}Pb/^{207}Pb$ ratios were predicted to be approximately 1.16.

Results

Pb in Soft Tissue and Shell

Because of its rapid turnover in the soft tissues, a principal determinant of Pb concentration is the snail's recent feeding activity, governed in turn by prevailing weather. Here populations were sampled within 1 week, at the end of an extended drought when there would have been little feeding. Thus their recent exposure history was likely similar.

Even so, Pb concentrations in soft tissues of snails collected at the various sites varied by several orders of magnitude. At

Table 2. Soft tissue and shell Pb concentrations (μg/g dry wt) and shell isotopic ratios

Site	Soft tissue concentration[a]	Shell concentration[b]	Shell Pb isotopic ratios[c]		
			$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{206}Pb/^{207}Pb$
BE	3.8 (148.8)	5.0	18.608(19)	15.512(20)	1.200
BI	411.8 (407.3)	2.5	17.122(21)	15.441(16)	1.109
CA	1.6 (15.9)	0.3	18.142(31)	15.449(34)	1.174
CN	5.3 (7.0)	2.4	17.894(2)	15.548(2)	1.151
CR	8.5 (28.3)	2.4	17.842(14)	15.511(10)	1.150
ET	<DL (6.0)	2.5	17.675(14)	15.482(12)	1.142
FB	2.5 (14.9)	2.3	18.272(30)	15.578(28)	1.173
FR	20.0 (14.4)	1.2	17.502(7)	15.493(6)	1.130
GS	16.5 (15.6)	5.3	17.934(11)	15.526(11)	1.155
HA	150.0 (131.6)	17.1	18.830(14)	15.518(12)	1.213
HW	<DL (22.7)	1.0	17.322(31)	15.450(32)	1.121
ID	52.3 (235.5)	8.1	17.090(6)	15.425(9)	1.108
IF	<DL (0)	0.4	17.525(17)	15.417(16)	1.137
IR	2.8 (8.1)	1.1	17.629(10)	15.442(11)	1.142
KB	13.6 (13.1)	8.3	17.718(9)	15.501(12)	1.143
LE	<DL (2.3)	0.6	17.468(3)	15.484(4)	1.128
LK	87.5 (100.7)	5.2	17.330(8)	15.480(7)	1.120
LL	<DL (0)	1.1	17.141(6)	15.383(6)	1.114
LU	38.0 (19.9)	1.1[d]	17.257(20)	15.513(19)	1.112
LW	70.0 (91.8)	5.7[d]	17.898(12)	15.547(26)	1.151
MI	21.6 (27.6)	13.3	18.186(10)	15.538(10)	1.170
NC	107.5 (118.3)	14.4[d]	17.775(13)	15.512(13)	1.146
NS	11.8 (86.1)	1.7	17.472(16)	15.398(14)	1.135
RD	<DL (28.6)	1.6	17.531(17)	15.435(15)	1.136
SA	10.3 (12.8)	2.6	17.653(16)	15.442(17)	1.143
SB	9.5 (424.4)	4.6	17.438(11)	15.485(9)	1.126
SC	7.8 (5.8)	6.4	19.675(10)	15.492(8)	1.270
SF	19.2 (13.7)	1.9	17.517(7)	15.508(10)	1.130
SH	127.6 (119.6)	6.6	17.936(19)	15.550(17)	1.153
SJ	35.8 (31.1)	3.2	17.317(9)	15.494(9)	1.118
ST	32.5 (42.0)	4.8	17.587(5)	15.462(4)	1.137
SU	11.9 (7.1)	2.5	17.452(13)	15.628(11)	1.117
TO	4.9 (10.9)	1.7	17.037(10)	15.286(11)	1.114

[a] <DL denotes less than detection limit. Five snails were analyzed separately (N = 5). Expressed as median with the interquartile range noted in parentheses

[b] Precision of shell Pb concentrations was approximately 5% as determined by replicate analyses of NIST SRM 277, 1646 and 1633a

[c] Errors are noted as 1 standard error of the mean and occur in the last decimal place(s), *e.g.*, 19.100(10) = 19.100 ± 0.010; $^{206}Pb/^{207}Pb$ ratio derived from the other ratios

[d] Median value for this shell sample with N > 1

Gravelly Hill (BI), the site of a major highway interchange, the median concentration exceeded 400 μg/g dry wt (Table 2). In contrast, soft tissues in snails taken from several rural sites (*e.g.*, LL) had Pb concentrations below the procedural detection limit. Despite the wide variation among snails from each site as reflected in the interquartile range, the median concentrations were judged to grossly reflect the level of Pb contamination at each site. This judgement was based on the observation that, although there was very wide variation among snails at each site due to the high Pb turnover rates in soft tissues, this variation at most sites was associated with a skewed distribution with one extremely high individual. For example, the most extreme variabilities were noted for the BE, ID, and SB sites. In two of these extreme cases, the wide interquartile range was strongly influenced by only one of the snails analyzed. The Pb concentrations were 1.5, 3.0, 3.8, 10.5, and 291.8 μg/g dry wt for the BE samples. Those for the SB samples were 5.5, 7.8, 8.3, 10.8, 26.5, and 1257.5 μg/g dry wt. However, PB concentrations in ID soft tissue samples (5.8, 8.7, 52.3, 184.6, and 300.9 μg/g dry wt) were not strongly influenced by a singleton. Shell Pb concentrations ranged from less than 1 to slightly more than 17 μg/g dry wt. Variation among shells from each site was lower than the variation among soft tissues as indicated by the calculated coefficients of variation for sites LW (37%) and NC (75%).

Pb Isotopic Signature

The general age and relative amount of radiogenic Pb in the Pb sources are shown in Figure 2 based on data from shells. The $^{206}Pb/^{207}Pb$ ratio would be highest in the geologically younger, native sources and the $^{206}Pb/^{204}Pb$ ratio would be highest in the more radiogenic, native sources of Pb. Shells from sites BE, CA, FB, HA, MI, and SC showed a clear predominance of native Pb (Figure 2). Sites BE, MI, and SC were in areas with long histories of Pb mining. HA was downwind of the Avonmouth smelter within an area with a long history of Pb working

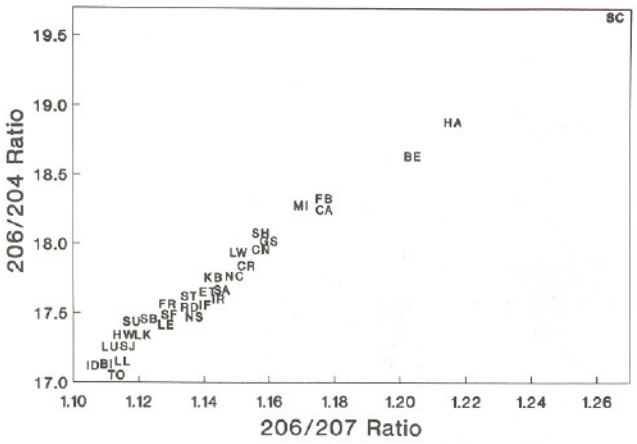

Fig. 2. $^{206}Pb/^{207}Pb$ versus $^{206}Pb/^{204}Pb$ plot for shells from the 33 sample sites. The $^{206}Pb/^{207}Pb$ generally reflected the geological age and $^{206}Pb/^{204}Pb$ reflected the relative amounts of radiogenic and nonradiogenic Pb of the source

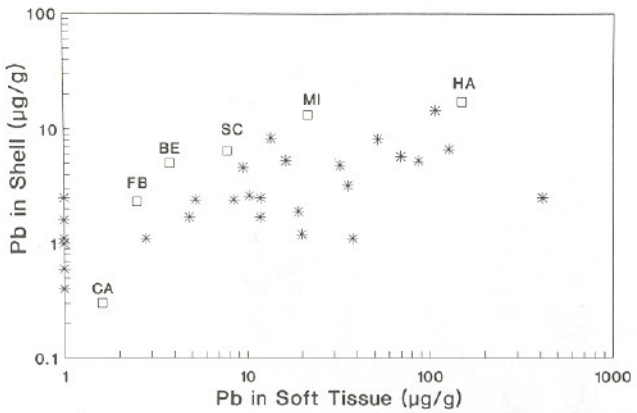

Fig. 3. Lead concentrations in soft tissues (medians) and shells of snails from sites dominated by native Pb sources (squares) or from non-native and mixed Pb sources (asterisks)

(Martin *et al.* 1979). The lack of any significant automotive sources or high levels of native sources was reflected in the low Pb concentrations with a native Pb signature for the CA site. Although site FB was near a parking lot, the low Pb concentrations and isotopic ratios indicated a dominance of native Pb. Assuming ratios decline with increasing proportion of automotive Pb, composition of shells from most of the remaining sites suggested a mixed origin for the Pb. Inputs from the highways next to the BI and ID sites were reflected in the high Pb concentrations and distinctive Pb isotopic ratios. The isotopic ratios noted for site TO accurately indicated that the site, an abandoned garage yard, received much Pb from automotive sources. Sites SB, SF, SJ, and SU were all close to each other in the suburbs of London and had very similar isotopic ratios reflecting significant automotive sources. Although LL was in a Pb rich area near sites BE, LW, and MI, its Pb isotopic signature clearly indicated that most of the shell-associated Pb was from the nearby road.

The exposure duration for the *Helix* populations was implied to be very long for the sites dominated by native Pb (BE, CA, FB, HA, MI, and SC). Estimation of exposure duration for other sites was complicated by the observed mixture of Pb sources. Combining these two exposure duration classes with tissue Pb concentrations provided a general depiction of the intensity (Pb concentration) and duration of exposure for the surveyed populations (Figure 3).

Discussion

The unique isotopic signatures of native Pb and those derived from the non-native anthropogenic sources provided the means for identifying Pb source and implying general duration of population exposure. Interpretation of these ratios was consistent with the known history of mining or smelting activities in the surveyed areas. The isotopic ratios were especially important for samples from areas with both a long history of Pb working and potentially significant, recent sources of Pb.

Prior this survey, we hypothesized that Pb concentrations in shells and soft tissues would change disproportionately if selection were occurring for sequestration of Pb in the shell. The realized shift would be influenced by exposure intensity and duration. Lead concentrations in shells and soft tissues of snails from sites with long exposures (native Pb) to high Pb concentrations (Figure 3) provided inferential support for this hypothesis. Except for samples from site CA, snails from populations with long histories of exposure (millennia) to high Pb levels had proportionately more Pb in their shell than soft tissue compared with snails from other surveyed populations. The CA site was unique in having native Pb at very low concentrations, thus selective pressure at this low Pb site could have been insufficient, despite the long exposure duration, to produce a measurable shift in the shell:soft tissue distribution of Pb. Despite the high concentration of Pb at Gravely Hill (BI), the duration of exposure was comparatively short and snails did not show an increased capacity to add Pb to the shell.

Physiological adaptation cannot be rejected as an alternative mechanism. Unfortunately, the genetic basis for differences between populations often remains ambiguous in such studies (Posthuma and Van Straalen 1993). Controlled breeding experiments including quantification of bioaccumulation for snails from the various populations would be required to test this hypothesis rigorously. It is our intention to collect additional snails from several of these sites for such studies.

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