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Metal Accumulation in Terrestrial Pulmonates at a Lead/Zinc Smelter Site in Arnoldstein, Austria

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Recently, Berger and Dallinger (1993) reviewed the suitability of terrestrial gastropods as quantitative indicator organisms for environmental metal pollution. The peculiar metal accumulation capabilities in molluscs have been known in detail for decades (e.g. Martin and Flegal 1975, Russell et al. 1981), but "only few data are available for terrestrial pulmonates" (Berger and Dallinger 1993). Furthermore, data are restricted to only a few species, and despite similarities in metabolic pathways, species-specific properties in metal budget strategies exist (Martin and Coughtrey 1982, Greville and Morgan 1990). Information concerning the potential range of metal burden in these animals from the field are, therefore, of ecophysiological relevance. Snails satisfy a basic demand as quantitative indicators of the bioavailable fraction of terrestrial metal pollution.

In general, most snail species can be regarded as "macroconcentrators" of certain metals (Cd, Zn, Cu), which refers to a biological concentration factor (ratio of assimilation constant and elimination constant) greater than 2 (Dallinger 1993). For example, *Helix pomatia* accumulated Cd even in unpolluted environments at higher levels than other invertebrate groups (Knutti et al. 1988).

In the present study, concentrations of Pb, Cd, Cu and Zn were measured in tissues of *Bradybaena fruticum* (Bradybaenidae), *Aegopis verticillus* (Zonitidae), *Arianta arbustorum* (Helicidae), and *Helix pomatia* (Helicidae). Animals were collected in the vicinity of a Pb/Zn smelter with a long history of pollution (in operation since 1495) in Arnoldstein, Austria. Metal concentrations in soil, plants and a variety of arthropod species at contaminated sites of this area have been reported elsewhere (Rabitsch 1995a, b, c).

MATERIALS AND METHODS

Snails of uniform size classes (10-15 per species) were collected at 3 sampling sites (Fig. 1) in the vicinity of the Pb/Zn smelter in Arnoldstein, Austria.

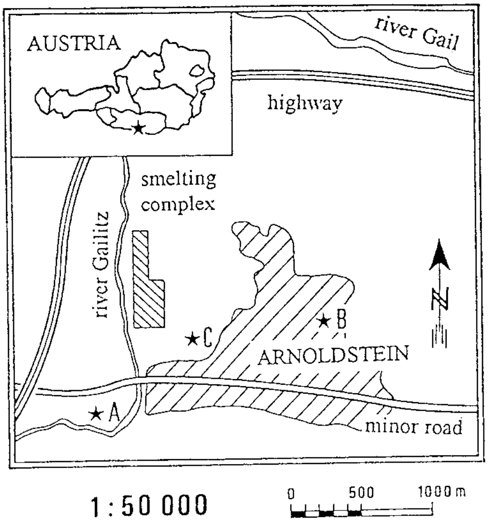

Figure 1. Location of the sampling sites (A, B, C) in the vicinity of the smelting complex in Arnoldstein, Austria.

Total amounts of metals in the soil were measured by flame atomic absorption spectrophotometry (VARIAN SPECTR AA30 with deuterium lamp background correction). Soil core samples (0-10 cm) were oven dried (105°C to constant weight), sieved (2-mm mesh size) and pulverized in an electric mill. Homogeneous aliquots were subjected to digestion in open teflon vessels with Aqua regia (140°C for 14h), finally diluted with double-distilled water (Table 1). Specimens of *B. fruticum* were collected at site B, *A. verticilllus* at site A only, and *A. arbustorum* at sites A and B, all on April 26, 1992. Adult *H. pomatia* samples were collected on the same date at the sites B and C, juveniles on September 17 1991 at sites A and C. The snails were placed in clean plastic containers at 4°C over two days for defecation, sacrificed, dissected, and divided into foot (F), hepatopancreas (lobe 1-3) (H) and kidney (K). The tissues were rinsed in deionised water, dried to constant weight (60°C for 24h), digested with concentrated nitric acid - either in open teflon vessels (140°C for 12h) or in closed polypropylene tubes (70°C for 4h) - and diluted 1:1 with double-distilled water. Elemental measurements were carried out by flame AAS as above. Simultaneous performance of analytical blanks and standard reference material (TORT-1, Lobster Hepatopancreas, Nat. Res. Council Canada) confirmed that the accuracy of methods was within acceptable limits.

Since data deviate from normal distribution (Kolmogorov-Smirnov test), nonparametric statistical techniques were carried out. Differences of metal concentrations between the three tissues were tested using the Kruskal-Wallis test. Comparison between two samples (site, age, tissue) was made applying the Mann-Whitney U-test for each metal.

RESULTS AND DISCUSSION

The aerial dispersion of the metal pollutants emitted by this point source followed prevailing winds and was responsible for the preferred easterly direction of extension. Accordingly, site pollution (measured as total soil metal contamination) increased in the order: site A - B - C (Fig. 1, Table 1).

Table 1. Locative and descriptive parameters of sampling sites.

Site	Distance from smelter (m)	Direction	Average metal concentrations (mg/kg) in soil (Aqua regia-fraction, 0-10 cm)			
			Pb	**Cd**	**Cu**	**Zn**
A	700	SW	347	8.9	54.0	1187
B	1000	E	828	11.4	62.9	1212
C	250	SE	2440	23.6	100.2	2743

Lead, Cd and Zn concentrations of *B. fruticum* differed significantly between tissues ($p<0.001$), whereas copper did not (Fig. 2). The hepatopancreas showed the highest metal levels of all snail species in this study (Median Pb:454, Cd:300, Zn: 11854 µg/g dry weight). Significant tissue-specific differences were found in A. *verticillus* between the foot and hepatopancreas and kidney for Pb, Cd and Zn ($p<0.05$) (Fig. 2). No differences were observed between kidney and hepatopancreas (for all elements) or for copper (between all tissues). Tissue-specific metal accumulation patterns were found in A. *arbustorum* at both sites. The hepatopancreas appeared to be the main storage site of Pb, Cd and Zn, whereas most copper was found in the kidney (Fig. 2). The expectation of higher metal levels in specimens from site B versus site A failed for foot- (except Pb) and kidney-samples (except Cd) and for Zn levels, but was confirmed considering Cd and Cu in hepatopancreas samples. As found for *B. fruticum* and *A. arbustorum*, the hepatopancreas of *H. pomatia* appeared to be the main storage site of Pb, Cd and Zn, followed by the kidney and the foot, whereas Cu concentrations were more evenly distributed between the analysed body compartments (Fig. 3). Metal concentrations in adults exceeded those of juveniles (both from site C) in most cases. Among juvenile tissues, only Pb and Cd in the kidney were higher at the more polluted site, whereas in adults the Pb, Cd and Zn concentrations in all tissues (and even Cu in the hepatopancreas) reflected this degree of site pollution.

The quantitatively important role of the hepatopancreas as the main storage organ of Pb, Cd and Zn was found previously in snails from the field (e.g. Cooke et al. 1979, Coughtrey and Martin 1976, Williamson 1980) as well as, in laboratory experiments (e.g. Dallinger and Wieser 1984). This was also confirmed by the present results on *B. fruticum, A. arbustorum* and *H. pomatia.* In contrast, Cu is usually more evenly distributed in the mollusc's body because it is associated with haemocyanin and is stored in cells surrounding the blood vessels (Moser and Wieser 1979, Mason et al. 1984).

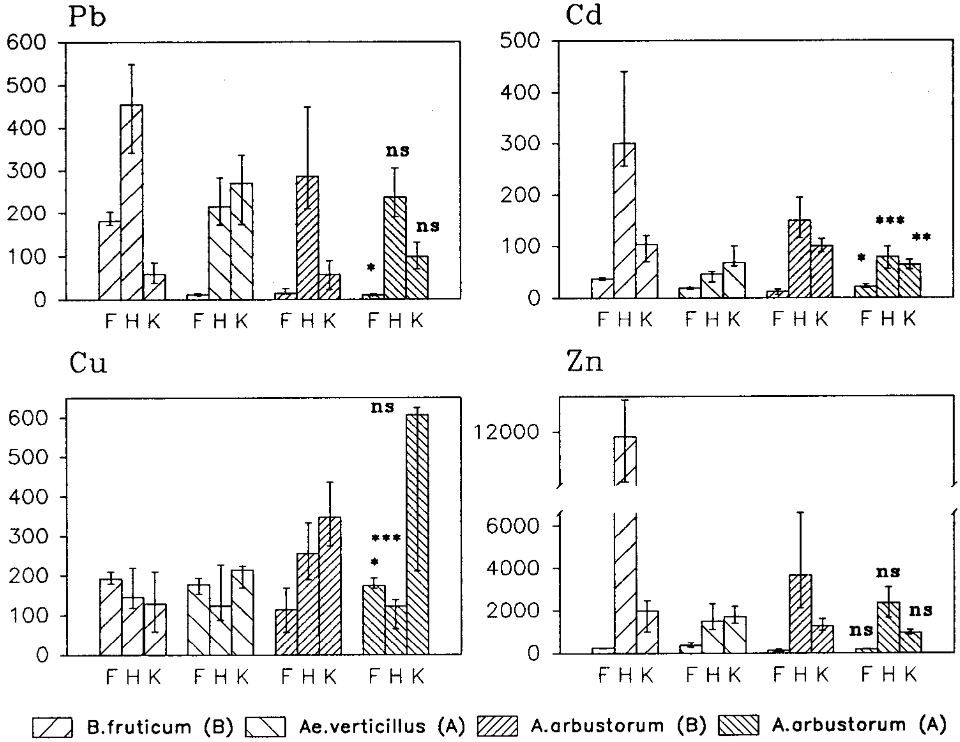

Figure 2. Median (µg/g dry weight) (bar) and quartiles of metal concentrations in tissues of three snail species. Sampling sites are given in parentheses: Foot (F), Hepatopancreas (H), Kidney (K). Asterisks above the bars signify site-specific differences of *A. arbustorum* tissues between site A vs B as follows: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = $p > 0.05$.

Although similar tissue-specific metal accumulation patterns occur between the analysed species, such a specific pattern depends on the species-specific properties in dealing with excess metals in the environment. Therefore, the accumulation of Pb, Cd and Zn in the kidney of *A. verticillus* and of Cu in the kidney of *A. arbustorum* and *H. pomatia*, matching the concentrations of the hepatopancreas, is of special interest. Perhaps the accumulation capabilities in the hepatopancreas have become exhausted and the kidney is then “flooded” by the excess of metals. The binding capacity of metallothioneins (low-molecular weight, metal-specific binding proteins with typical amino acid composition and conservatively repeated tripeptide sequence) may have become saturated and the animals forced to switch to other strategies in elemental maintenance, A higher metal output by excretion instead of storage may be a such a strategy, the consequence being metal accumulation in the kidney (organ of excretion), or at least higher transient times during metal excess.

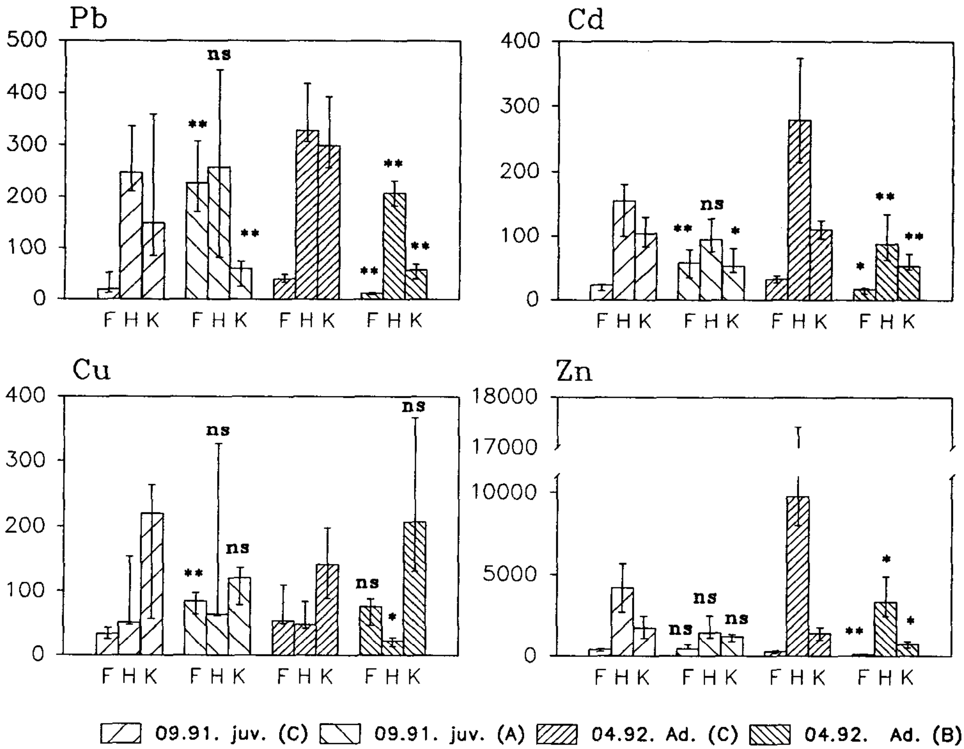

Figure 3. Median (μg/g dry weight) (bar) and quartiles of metal concentrations in tissues of *Helix pomatia.* Sampling sites are given in parentheses: juveniles (juv.), Adults (Ad.). Foot (F), Hepatopancreas (H), Kidney (K). Site-specific differences C versus A (juv.) and C versus B (Ad.) are given above the bars. Asterisks denote statistical differences as in Fig. 2.

Correspondingly, the amount of Pb and Zn in the kidney was found to increase with the degree of contamination for *Helix aspersa* (Coughtrey and Martin 1976). The saturation of detoxifying protein sites in the hepatopancreas mentioned above can also cause a so-called "spillover effect" (Janssen and Dallinger 1991), resulting in the binding of metals to additional components. A comparison of tissue metal levels between species at the same site revealed pronounced differences, corroborating that metal concentrations of snails is a function of species-specific metal kinetics. The expectation of higher metal levels with increasing age is confirmed by the present results of *H. pomatia:* adults exhibit higher metal levels than juveniles at the same site. Similarly, a sharp increase of Cd concentrations at the time of maturation was found in *H. pomatia* by Knutti et al. (1988). A site-specific metal accumulation pattern, based on site metal pollution, was found in hepatopancreas samples of *A. arbustorum* (Fig. 2) and adult *H. pomatia* (Fig. 3). A rather indifferent pattern was found in other tissues and in juvenile *H. pomatia.* The higher metal tissue burdens found at sites closer to the emission point source correspond to established criteria of quantitative indicator organisms (Arndt et al. 1987), even between adjacent populations. Juveniles, as well as, foot and kidney samples of adults are of limited value for monitoring purposes. Whereas Pb and Cd concentrations in the hepatopancreas were only slightly higher compared with literature data, Zn and Cu levels exceeded previously reported values for snails from contaminated field sites by factors of 6 and 2 to 4, respectively (Hopkin 1989). It is worth mentioning that Pb levels in the snails were not exceptionally elevated above literature levels, even though Pb is the dominant pollutant of the region (Rabitsch 1995a).

The use of snails as bioindicators has been discussed earlier (Meincke and Schaller 1974) and the dependence of accumulation patterns on biological variables (body size, temperature, season) has been determined (Meincke and Schaller 1974, Coughtrey and Martin 1977, 1982, Williamson 1979, 1980). Definitions of background levels as well as "upper" limits of possible body burdens in the field are recommended in applying a particular species in a biomonitoring scheme. One major goal of using bioindicators is to possess the sensitivity to detect recent or less polluted sites. Consequently, the use of the hepatopancreas of adult snails, instead of whole animal samples, is suggested for monitoring purposes. The hepatopancreas of adult snails, serving as a storage site for excess body metal levels and as a sink for metal detoxification pathways, may serve as a better and more sensitive indicator than whole animal samples, particularly in less polluted environments.

Although the high metal levels in snail tissues in the vicinity of the smelter site in Arnoldstein can be expected and are not surprising, the present body of data contributes to our knowledge about the metal accumulation potential of terrestrial pulmonates in the field and proposes a more sensitive approach to monitoring metal pollution.

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