ARION ATER (MOLLUSCA: PULMONATA) AS AN INDICATOR OF TERRESTRIAL ENVIRONMENTAL POLLUTION

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Abstract. Using X-ray energy spectroscopy concentrations of Mn, Fe, Cu, Zn, As, Br, Pb, Rb, and Sr were measured in specimens of the terrestrial gastropod, *Arion ater*, found in locations close to and far from a highway. Higher values of Pb and Br were observed for specimens near the polluting source and mathematical analysis revealed an inverse relationship between lead uptake and uptake of Cu, Zn, and Sr. The data are used to demonstrate a classification procedure in which trace metal concentrations in *Arion ater* are used to assess environmental quality.

1. Introduction

The slug, *Arion ater*, is a common terrestrial mollusc of south-western British Columbia, and may be useful for monitoring the ambient levels of trace metals in the environment. Since it regularly returns to the same burrow after feeding within an area of only a few square meters (Rollo and Wellington, 1977), the levels of trace metals within *Arion ater* potentially represent a time-integrated and space-integrated estimate of primarily aerial trace metal pollution of its immediate terrestrial environment so that variables such as rain washing of leaves, incidental contamination of the soil in a very small area and unequal uptake of trace metals by plants living in the sampled area would be minimized. Further, *Arion ater* appears to occupy both undisturbed (rural) habitats and disturbed (urban(habitats and thus could be found in locations of either low or high ambient levels of trace metals. Thus the same species can be sampled from different environments avoiding problems due to differences in trace metal uptake by different species of slugs or other gastropod molluscs.

Organisms are not exposed to just one elemental toxin at a time but rather to all simultaneously (Schubert, 1973), so that ideally the concentration of as many as possible of the trace elements should be determined. X-ray energy spectroscopy (XES), which measures energies and intensities of emitted X-rays from an irradiated sample, is such a method for performing multi-elemental determinations simultaneously (Goulding and Jaklevic, 1973). Further advantages of XES include simple

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sample preparation, non-destructive and rapid analysis of the prepared sample, and minimum sample handling, thus reducing contamination effects.

Since there are high levels of Pb in the soil, litter, plants and animals collected near roads (Cannon and Bowler, 1962; Gish and Christensen, 1973; Lagerwerff and Sprecht, 1973; Welch and Dick, 1975), collection of slugs from three different proximities to a well travelled highway (30 000 cars per day) and determinations of trace elemental concentrations in them by XES was a practical method of monitoring pollution in a terrestrial system. Such an analysis would help to show if trace elemental concentrations varied in slugs collected from different habitats and reveal if there were any inter-elemental effects.

2. Materials and Metholds

Six specimens of the slug *Arion ater* were collected from each of the following locations: 0 to 40 m from a well-travelled highway, 40 to 80 m from the highway, and near the top of a hill 800 m high and located about 3.5 km north of the highway. The two locations near the highway were separated by a creek thus making a natural boundary to prevent mixing of the two populations. All specimens were collected between mid-August and mid-September (1977).

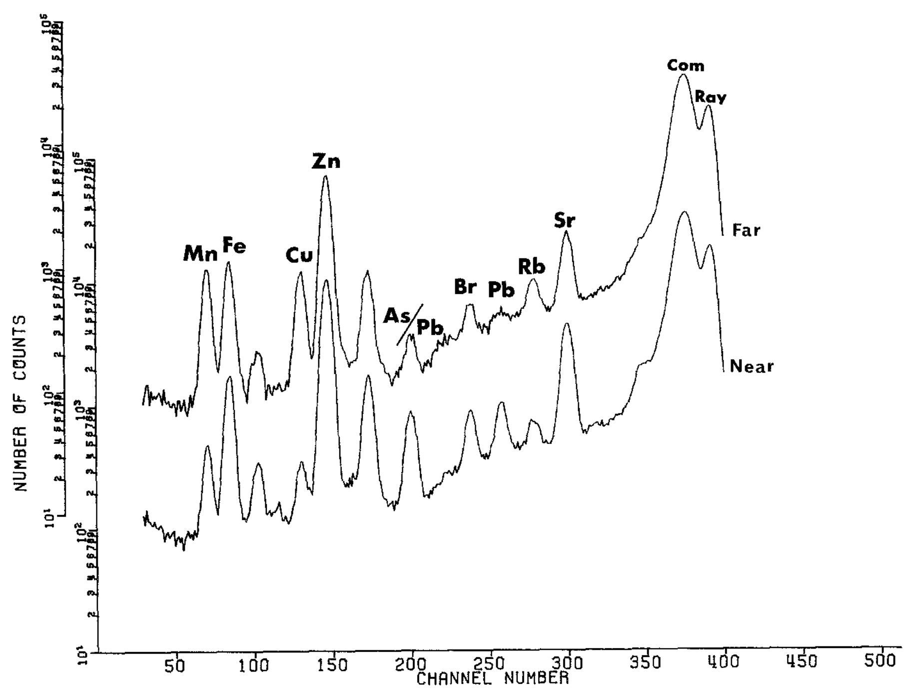

Fig. 1. X-ray spectra of pellets prepared from *Arion ater* collected from sites near and far well-travelled highway. Various elemental peaks have been labelled. The Compton (C) and Rayleigh (R) scatter peaks from the incident Mo X-ray radiation are also indicated.

The slugs were weighed (fresh weight), frozen in dry ice, cut transversely in half and freeze dried in plastic bottles (SPEX Industries). They were then chopped into smaller pieces, pulverized into a powder 80 mg of which were then pressed into a self-supporting pellet, and finally analysed using XES, a description of which is given by Stump *et al.* (1977).

The resulting spectra (Figure 1) were analyzed for energies and areas of the observed peaks using the computer program SAMPO (Routti and Prussin, 1969). These areas were then normalized to the Mo incoherent backscatter (Compton) peak (Giaque *et al.*, 1977). Coefficients for converting the normalized peak intensities to trace element concentrations in the samples were obtained by using the method of standard additions in samples of the same chemical composition (Stump *et al.*, 1979). A correction factor for the interference of the Pb L_α line with the As K_β was obtained by determining empirically the L_α/L_β ratio for Pb in a sample of similar absorption effects (Stump *et al.*, 1979). The observed peak (labelled As/Pb in Figure 1) was then corrected using the L_β Pb peak (labelled Pb in Figure 1) to extract the true As contribution. All coefficients were confirmed by analyses of NBS standard samples (SRM 1569 and SRM 1571) and were found to give concentrations within 10% of the true values. A further verification for Pb was obtained by atomic absorption spectrophotometry of six powdered samples of the same slugs.

3. Results

Table I lists the arithmetic means, standard error of the means, and geometric means of the body weight and concentrations of trace elements in the slugs collected from the three habitats. Table II lists the concentration of lead in slugs as determined using

TABLE I

Arithmetic means ($\bar{x}$), ± standard error of the means (*SE*) and geometric means ($\bar{g}$) of the concentrations (ppm) of trace elements found in *Arion ater* collected from three localities: near a well travelled highway (Hgy-near), 80–120 m from the highway (Hgw-far) and from a location 1 km away from the highway (Park). Statistics of wet weights are given also.

Element	Hgy (near)		Hgy (far)		Park	
	$\bar{x} + SE$	$\bar{g}$	$\bar{x} + SE$	$\bar{g}$	$\bar{x} + SE$	$\bar{g}$
Mn	1286 ± 413,	935	608 ± 108,	549	485 ± 95,	434
Fe	921 ± 123,	879	500 ± 125,	432	481 ± 136,	414
Cu	67 ± 18,	61	103 ± 10,	101	111 ± 14,	105
Zn	647 ± 186,	507	592 ± 60,	575	882 ± 106,	846
As	2 ± 1,	2	4 ± 1,	4	3 ± 1,	1
Br*	107 ± 20,	101	87 ± 7,	85	22 ± 2,	21
Pb*	162 ± 30,	148	20 ± 2,	20	23 ± 7,	18
Rb	16 ± 2,	15	20 ± 2,	20	35 ± 12,	26
Sr*	206 ± 22,	200	335 ± 42,	324	132 ± 32,	118
Wet weight	5.9 ± 1,	5.5	7.2 ± 0.6,	7.0	12.2 ± 2.3,	12.1

* These elements display statistically significant ($P = 0.05$) variation as a function of location.

TABLE II

Comparison of concentration of Pb in four specimens of slugs as determined by X-ray energy spectroscopy and atomic absorption spectrophotometry*, respectively

Specimen	XES	AAS*
NP3P	11.9 ± 0.7	16
NP4P	4.2 ± 0.3	13
SF4P	22.9 ± 0.5	24
SF5P	14.2 ± 1.2	25

* Concentration measured at City Analayst's Laboratory of Vancouver.

both atomic absorption spectrophotometry and WES. The matrix of the correlations among the variables is shown in Table III. The results of a principal component analysis of the correlation matrix is listed in Table IV.

The data were log-transformed and subjected to an analysis of covariance (body weight as the covariate) to discover if statistically significant differences ($P = 0.05$) existed among the means of each element. Those elements found to have significantly different means are marked with an asterisk in Table I. These results show that slugs collected from the roadside have significantly higher concentrations of Pb and Br than those collected further away. Slugs collected 80 m beyond the highway edge were found to have significantly higher concentrations of Sr. The trends of decreasing mean concentrations of Mn and Fe in the slugs in relation to proximity to the highway were not found to be significant. This may be due in part to the small sample sizes used as there is some evidence that Mn can be found in elevated concentrations near roadsides (Pierson *et al.*, 1978). The ranges of concentrations of Cu, Zn and Pb in as found in this study in British Columbia are similar to those found for *Arion ater* in Wales (Ireland, 1979), for *Helix aspersa* (Coughtrey and Martin, 1976, 1977), and for *Arion hortensis* and *A. fasciatus* (Martin and Coughtrey, 1976). With respect to the verification studies, the values obtained by XES are consistently lower than those obtained by A.A.S., suggesting that estimations of Pb concentrations are not overestimated.

Many correlations among the log-transformed variables (Table III) are statistically significant ($r = 0.4$ at $P = 0.05$ with 16 degrees of freedom) prompting the question as to whether there are some 'underlying factors' or components that are 'controlling' these variables. One method of finding such structure in the data is to apply a principal component analysis of the correlation matrix. Principal component analysis (Cooley and Loh nes, 1971; Kendall, 1975) reduces the high number of original correlated variables to a few composite uncorrelated variables (components). Each of these variables accounts for successively smaller proportions of the total variance. In this study the routine used for finding the principal components was from the BMDP statistical package (Dixon, 1977). The technique is heuristic rather

TABLE III

Correlation matrix of the variables after log transformation

		Wet wt. 1	Mn 2	Fe 3	Cu 4	Zn 5	As 6	Br 7	Pb 8	Rb 9	Sr 10
Wet weight	1	1.000									
Mn	2	−0.358	1.000								
Fe	3	−0.384	0.276	1.000							
Cu	4	**0.439**	−0.385	−0.279	1.000						
Zn	5	**0.430**	−0.205	−0.143	**0.684**	1.000					
As	6	−0.192	0.000	0.096	0.127	0.125	1.000				
Br	7	**−0.665**	0.243	**0.410**	−0.272	−0.336	0.335	1.000			
Pb	8	**−0.441**	0.262	**0.563**	**−0.512**	−0.298	**−0.347**	**0.528**	1.000		
Rb	9	0.297	−0.102	−0.307	0.174	0.201	−0.170	**−0.314**	−0.314	1.000	
Sr	10	**−0.453**	0.235	0.051	−0.134	−0.053	**0.511**	**0.606**	−0.001	−0.101	1.000

Bold numbers show statistically significant correlations ($P = 0.05$).

TABLE IV

Correlations of three principal components with the body weight and trace metal concentrations after log transformation

Variates	Components 1	2	3	Communalities
Wet weight	−0.811	−0.153	0.111	0.693
Mn	0.508	−0.075	−0.256	0.330
Fe	0.605	−0.108	0.526	0.654
Cu	−0.674	0.414	0.400	0.786
Zn	−0.505	0.354	0.465	0.685
As	0.151	0.853	−0.012	0.751
Br	0.812	0.351	0.129	0.800
Pb	0.695	−0.459	0.346	0.814
Rb	−0.498	−0.091	−0.461	0.469
Sr	0.464	0.701	−0.277	0.784
Eigenvalues	3.709	1.899	1.157	
% proportion of variance	37.1	19.0	11.5	

than inferential so that concepts related to significance testing are not necessary.

As shown in Table IV three components with eigenvalues greater than 1 (the usual cut-off point for interpretation; Kendall, 1975) account for 67.6% of the total variance of the data. The first component, accounting for 37.1% of the variance, has high correlations with the weight of the slugs and the concentrations of the trace elements, suggesting that it is identifying the relationship between size and elemental concentration. The bipolar nature (having both positive and negative correlations) of the component indicates that as size increases in the slugs the concentrations of Cu and Zn increase but the concentrations of Pb and Br decrease. This apparent inverse relationship occurring between Cu and Zn concentrations, and Pb and Br concentrations, raises the question as to whether high Pb and Br concentrations in slugs result in a decrease in their size and inhibition of Cu and Zn uptake. In other words, it appears that this component may be identifying a toxic effect of Pb (and perhaps Br) exposure as well as showing a functional relationship between size and trace element concentration.

The second component accounting for 19.0% of the variance is highly positively correlated with Sr and As concentrations and negatively correlated with Pb concentrations. Two interpretations (not necessarily mutually exclusive) of these correlations with this component are possible. The first is that there is an interaction between uptake or assimilation of Pb and Sr (an analogue of Ca) such that Pb either replaces Sr in the slug or else inhibits its uptake (and by implication, the uptake of Ca also). The second interpretation is that there is a systematic error in the determination of As concentrations since the value for As is obtained after subtracting the effect due to interference by Pb. If the effect of Pb interference is overestimated then the concentration of As will be consistently underestimated in proportion to the increasing values of Pb concentration. However, the peak area for the overlapping As ana Pb lines was invariably close to the peak area of the Pb L_β line, suggesting that the concentration of As in the slugs may have been near the lower limit of detection for the XES system. This implies that not too much significance should be placed on this second interpretation with respect to Pb and As.

The third component, accounting for only 11.5% of the variance, is difficult to interpret since it is highly positively correlated Fe, Cu, Zn and Pb concentrations, and negatively correlated with Mn, Rb and Sr concentrations. Since such a small fraction of the variance is accounted for by this component and since the sample size is small it is best to leave it uninterpreted.

4. Discussion

Use of molluscs as monitors of trace metal pollution both in aquatic and terrestrial environments can be criticized for three reasons. The first is that trace metal concentrations in molluscs are functions of the size of the molluscs (Coughtrey and Martin, 1977; Boyden, 1977); the second is that the uptake of one or more trace elements is affected by the concentrations of trace metals already present in the mollusc (Martin and Flegal, 1975; Coughtrey and Martin, 1977); and the third reason (so far restricted to acquatic molluscs) is that trace metal uptake and therefore concentration is affected by environmental factors such as salinity (Phillips, 1976a, b; Davenport, 1977).

Nevertheless, despite the apparent weaknesses in using molluscs as monitors of trace element pollution, the results of this study do show that independent of differences in body weights, slugs collected close to a roadside have significantly higher concentrations of Pb and Br than those collected further away. Preliminary analyses of samples of soil and vegetations collected close to the highway also show high levels of Pb as would be expected (see Introduction). Thus it would seem that slugs take up Pb in some proportion to that found in the environment and can be used for monitoring purposes. That slugs do reflect the ambient levels of trace metals in the habitat from which they are collected was also concluded in a recent study by Ireland (1979). The high concentrations of both Pb and Br in the slugs collected near the roadside strongly suggest that they are being exposed to the Pb–Br compounds of automobile emissions (Ter Haar and Bayard, 1971).

Principal component analysis of the inter-correlations of the variables has helped to point out where possible interactions among the variables may be occurring. The analysis has shown the tendency of the elemental concentrations to be a function of weight of the slugs, as they have been shown to be for other molluscs (Boyden, 1977; Coughtrey and Martin, 1977), and it has illuminated relationships, not otherwise readily apparent, between Pb uptake and the uptake of other elements, notably Cu, Zn and Sr.

An example of a toxic metal such as Pb influencing Zn uptake and growth in a gastropod has been reported previously by Yager and Harry (1964). They found that while low concentrations of Ca did not kill the freshwater snail, *Taphius*, it did reduce its ability to accumulate Zn. Hence Pb could be acting in a way seen in mammalian systems, by interacting with Ca and Sr systems and inhibiting the uptake of these two elements (Six and Goyer, 1970; Gruden *et al.*, 1974; Hsu *et al.*, 1975; Sorrel *et al.*, 1977).

5. Environmental Classification

If it is accepted that trace element concentrations in slugs reflect elemental concentrations in the environment, then data like those collected in this work can be used for monitoring and as a basis of classification of environmental quality of previously unsampled habitats. In other words it may not always be necessary to collect large numbers of organisms from many areas and perform analyses of covariance. Specimen collecting is both expensive and time consuming so that classification procedures for monitoring purposes may be appropriate.

For example, let us agree that slugs with high concentrations of Pb come from a Pb-polluted environment such as a roadside, and that slugs with low concentrations of Pb come from a non Pb-polluted habitat. Now given data on Pb concentrations in three concentrations in three hypothetical slugs, A, B and C (Table V) collected from three new hypothetical habitats it is possible to make an objective decision as to whether these slugs come from habitats polluted with Pb. Figure 2 shows the Pb concentrations of the three slugs (A, B, C) in relation to the t-distribution of the measured Pb concentrations in slugs forming the basis of the classification. Note that

TABLE V

Defining environmental quality of three hypothetical slugs

Type location representative of environmental quality	
Highway (near) (Pb polluted)	Highway (far) (not Pb polluted)
Log mean [Pb],	
x: 2.169	1.297
Standard deviation	
s: 0.213	0.116
Sample size	
n: 6	6

Hypothetical sample from habitat of unknown quality	Hypothetical [Pb] (ppm)	log [Pb]	χ^2	Pb polluted Probability of similarity $[1 - P(x_i\|\bar{x})]$	χ^2	Not Pb polluted Probability of similarity $[1 - P(x_i\|\bar{x})]$
A	19	1.28	17.42	0.004	0.02	> 0.999
B	35	1.54	8.72	0.121	4.38	0.496
C	93	1.97	0.88	0.972	33.66	< 0.001

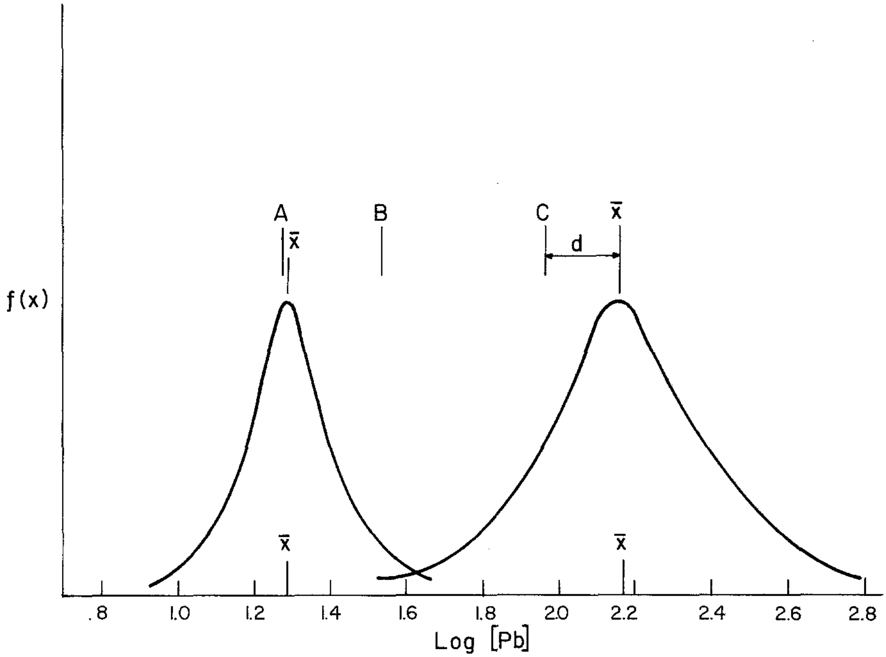

Fig. 2. *t*-distributions determined from measured Pb concentrations in *Arion ater*, forming the basis of environmental classification. The concentration in three hypothetical slugs (A, B, C) are indicated where *d* is the difference between the measured mean value, x̄, and 'observed' value of slug, C.

slug C is closest to the sample with the higher mean Pb concentration. Intuitively we can see that it is the most likely one to come from a habitat polluted with Pb. The estimation of probability of this classification is based on 1 minus the probability of the distance function, χ^2, defined as

$$\chi^2 = \frac{(x_i - \bar{x})^2}{s^2} \tag{1}$$

(Cooley and Lohnes, 1971) where x_i is the single observation of comparison, $\bar{x}$ is the mean of the group to which the object is being compared, and s^2 is the variance of the group. Table V lists the probabilities of the slugs being sampled from populations of slugs like those collected in this study. These results show that slug C with the highest concentration of Pb has a 97.2% chance of having been collected from a Pb-polluted habitat and has a low chance (0.4 to 12.1%) of having been collected from a non Pb-polluted habitat. Slugs A and B have better than 99.9% and 49.6% chances, respectively, of having been collected from non Pb-polluted habitats. From such results it would now be possible to decide with confidence from which habitats the slugs were collected and where detailed studies should be made, i.e., those habitats from which slugs B and C were collected.

The above has been used for illustrative purposes only. For a rigorous basis of environmental classification, the quality of the habitats would not be based on the trace element concentrations of just six slugs. Nevertheless, such procedures as have been described may help to make monitoring studies more useful in assessing the impact of man-made changes upon the environment.

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