INHERENT VARIABILITY IN METAL CONTENT OF *PATELLA ASPERA* (LMCK)

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Abstract

The present work adresses inherent variability in metal concentration in organisms, a major cause of metal fluctuations in the gastropod *Patella aspera*. This phenomenon was studied under different environmental conditions in the Saronikos Gulf. The results were used for the calculation of the minimum number of replicates of *P. aspera* samples that are needed to give a representative metal concentration in *P. aspera* population.

Key-words : metals, gastropods, bio-accumulation, Aegean Sea

Introduction

The variability in metal concentrations of marine organisms depends on many factors, either environmental (concentration of metals in sea water, temperature, salinity, dissolved oxygen, hydrology of the area, etc.) (1, 2), or purely biological (species, sex, age, reproduction stage, etc.) (2). Part of the variability that has not been attributed to the above factors is reported in literature as "inherent variability" (3, 4). Sometimes it is so important that it exceeds 100%. We encounter this phenomenon very often and it seems to be more frequent and stronger in contaminated areas (5). Because inherent variability is a factor influencing the estimation of the average concentration of metals in samples collected from a specified area, the determination of the optimal number of replicates is of major importance (6, 7). The present work aims to study the phenomenon of metal variability in the gastropod *Patella aspera* which is considered as a good pollution bioindicator (8, 9). This species which is primarily herbivorous lives on the coastal rocks and is cosmopolitan and abundant in Greek waters. At the same time this study aims to define the minimum number of specimens (replicates) needed to be collected to obtain metal concentrations representative of the population.

Methodology

In order to study the phenomenon of metal variability under different environmental conditions, four coastal localities (stations) along the north-east coast of the Saronikos Gulf were chosen for the sample collection. From each location 30 specimens of similar size (2.5 to 3 cm diameter) were collected and transported within an hour to the laboratory. There the soft parts were removed with a PVC knife, rinsed abundantly with distilled water and placed into PVC Petri dishes. Each individual was treated and analysed as a separate sample. Consequently the samples were lyophilised, homogenised in a porcelain mortar and digested with HNO_3 under pressure at 120°C for 12 hours.

The metals copper (Cu), nickel (Ni), chromium (Cr) and zinc (Zn) were determined by atomic absorption spectrophotometry using a VARIAN AA157 device. The above analytical methodology was tested by analysing the N°279 (*Ulva lactuca*) reference material of BCR. The results of this test are given in Table 1.

Table 1. Control of the analytical methodology

Metal	certified value	value found
Cu	51.2 + 1.9	46.49+1.35
Cr	26.0	22.39+1.49
Ni	40.0	44.22+1.32
Zn	313+8	269.4+4.6

The variability of metal bioaccumulation was studied graphically and by regression analysis and one way ANOVA after log-transformation of the results.

Results and discussion

The results of the chemical analysis (as average and ranges) expressed in μg/g dry weight are given in Table 2. Generally the levels of chromium and nickel in the present study are similar to published values while those for zinc are lower and that of copper higher (10-14). It is interesting to mention that the spatial distribution of metal bioaccumulation in limpets was statistically different ($P < 0.005$) in the four sampling localities. But in all localities, the bioaccumulation showed a high degree of variation that in some cases reached 80% (Table 2). This is mainly attributed to inherent variability in individual metal content since the specimens were of similar size and the regression analysis between metal content and diameter did not reveal any relationship ($P > 0.05$). In an attempt to show how this variability influenced

Table 2. Average concentrations of metals in *P. aspera* (in μg/g dry weight) and variability (coefficient of variation-%).

Station		Cu	Ni	Cr	Zn
1	AVG(SD	11.30 ± 1.97	9.96 ± 2.31	1.78 ± 1.12	44.47 ± 4.01
	range	7.24-17.24	5.70-15.13	0.40-4.67	37.76-55.57
	c%	17.40	23.24	62.84	9.02
2	AVG ± SD	9.48 ± 2.01	23.05 ± 6.91	8.42 ± 6.71	65.75 ± 14.06
	range	5.63-15.30	10.08-44.57	0.40-30.06	30.63-96.83
	c%	21.18	29.96	79.76	21.38
3	AVG ± SD	11.01 ± 2.16	30.36 ± 12.10	14.16 ± 6.27	59.57 ± 6.96
	range	6.48-15.63	5.50-53.74	3.50-36.21	45.36-76.19
	c%	19.59	39.87	44.28	11.68
4	AVG ± SD	11.20 ± 1.97	19.06 ± 5.02	6.63 ± 4.37	60.12 ± 8.89
	range	7.70-14.72	10.59-30..39	0.88-17.97	42.72-87.29
	c%	17.55	26.33	65.95	14.79

the accuracy of the estimated average value of metals in the populations of *P. aspera*, we calculated (for each sampling station) the mean concentration of metals using a consecutively increasing number of samples (from 2 to 30). The results of these calculations are presented graphically in Figure 1. It is obvious that the left portion of the graphs – where the number of samples is low – shows a significant fluctuation and the calculated mean differs from that derived from 30 samples. It is also evident that it differs from the real mean concentration ($\bar{i}$) of the population. The phenomenon is especially marked for all metals in curves for station 2 and for copper and nickel in the curve for station 3. The optimal number of samples of limpets for bioaccumulation studies can be graphically determined from Figure 1: from the point where the curve becomes quite stable (15). In this case, it seems that 8 to 12 individual samples of limpets are sufficient for an accurate population estimate. In fact, the average of 8 to 12 values matches closely with those calculated from 30 samples (Table 3, Fig. 1).

Table 3. Summary statistics for bioaccumulation of metals in *P. aspera* depending on the number of samples/individuals (N) per sampling location.

Station		Cu		Ni		Cr		Zn	
	N	AVG	S. D	AVG	S. D	AVG	S. D	AVG	S. D
1	2	12.54	1.82	11.23	3.13	1.68	0.11	45.99	3.65
	12	11.25	1.72	9.70	2.16	1.45	0.81	44.11	3.34
	30	11.30	1.97	9.96	2.31	1.78	1.12	44.47	4.01
2	2	7.61	1.98	18.16	3.16	2.63	0.50	45.16	14.54
	12	9.91	2.36	24.32	6.89	8.21	7.31	62.64	15.42
	30	9.48	2.01	23.05	6.91	8.42	6.71	65.75	14.06
3	2	10.86	0.15	24.10	6.26	11.54	2.62	56.32	3.25
	12	11.09	1.58	24.28	14.42	14.79	7.94	60.59	4.95
	30	11.01	2.16	30.36	12.10	14.16	6.27	59.57	6.96
4	2	10.96	0.48	19.46	1.50	3.54	0.82	62.87	12.24
	12	11.05	1.44	17.50	3.43	5.49	2.54	61.20	10.97
	30	11.20	1.97	19.06	5.02	6.63	4.37	60.12	8.89

Alternatively to the graphical estimation is the mathematical estimate using the variance and the mean value from preliminary data and the acceptable error in the determination of the mean (15, 16). Using this method and with a 10% error, a variable number of samples is needed depending on the metal (Table 4). For Cu, Ni, and Zn, it seems that 8 to 12 samples are sufficient, while for Cr which displays a higher variability, the optimal number increases dramatically. However Puel *et al.* (6) have estimated a larger sample size (20 replicates) in order that an average concentration can be computed with a 5% error.