Heavy metals (Cd, Cu, Pb and Zn) in two species of limpets (*Patella rustica* and *Patella candei crenata*) in the Canary Islands, Spain

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Abstract

Nowadays, indigenous molluscs are being utilized more and more as biomonitors. Thus, in order to assess the concentrations of Cd, Cu, Pb and Zn in the Canarian Archipelago (Spain), we have utilized two species of limpets (*Patella rustica* and *Patella candei crenata*). We also tested the relationship between the metal levels and biometric parameters such as size and weight. The mean total concentrations of Cd, Cu, Pb, and Zn in *P. rustica* were 0.37 ± 0.05, 1.77 ± 0.09, 1.27 ± 0.07 and 8.84 ± 0.71 µg g^{-1} dry wt. (mean ± S.E.) respectively; whereas *P. candei crenata* as follows; 0.71 ± 0.10, 2.94 ± 0.11, 0.09 ± 0.01 and 33.74 ± 1.15 µg g^{-1} dry wt. (mean ± S.E.). We found significant differences for metal concentrations between the eastern islands and the western islands for both species. We did not observed evidences that more inhabited islands (Tenerife and Gran Canaria), had the highest metal concentration levels. Natural inputs from the upwelling region and other factors like size, food availability and/or excretion could have contributed to the variability. *P. rustica* presented negative correlations between metal concentrations and body weight and size, whereas, *P. candei crenata* showed negative correlations with Pb and with the biometric parameters. *P. candei crenata* revealed also positive correlation between Cd concentration and size.
Keywords: heavy metals, biomonitors, patella, Cu, Cd, Pb, Zn.

1 Introduction

Gastropod molluscs, principally mussels and oysters, have been largely utilized as biomonitor organisms [1, 2, 3] since they are known to concentrate metals better than many other invertebrates [4].

Mussels are rare [5] and oysters do not exist in the rocky coasts of the Canary Islands; however, other species of gastropods are extensively widespread such as limpets (*Patella* spp.) and topsnail (*Osilinus* spp.) [6, 7]. These gastropods are commonly harvested and consumed by humans [5, 8] and it could possibly be a harmful metal transfer to the trophic web.

Until now, no study has compared the metal levels of different species of molluscs throughout the Canarian Archipelago. Díaz *et al* [9] and Collado [10] studied metal concentrations in limpets or in topsnail but only in one island and without distinguishing between species or studying a unique species of limpet [11]. In our work, we have focused in the metal levels (Cu, Cd, Pb, and Zn) in two species of limpets (*Patella rustica* and *Patella candei crenata*) along the rocky coasts of the Canary Islands to found out heavy metal contaminations patterns in the different islands and the relations with the biometric parameters.

Figure 1: Location of sampling sites where samples of molluscs were recollected along the rocky coasts of the Canary Islands.

2 Material and methods

2.1 Study area and sampling

Our study was localized in three sites of each ones of the seven Canary Islands (13-19º W, 27-30º N) and in small islets that constitute the Chinijo Archipelago, in march 2003 (fig. 1). Organisms were randomly handpicked from each site according to their availability. Samples (*P. rustica*, n= 104 and *P. candei crenata*, n= 121) were placed in polypropylene bags and transported to the laboratory where each individual was measured (total length), rinsed with deionized water (Milli-Q, Millipore, 18 MΩ·cm), and frozen in new bags until the digestion processed [12]. Only plastic materials like polypropylene, polyethylene and Teflon® previously treated [12] (bags, digesters beakers, flasks, bottles or tweezers) were in contact with the samples.

2.2 Analysis

The soft tissues of the molluscs were carefully extracted, cleaned and dried to constant weight under an IR-light lamp. Samples digestion was carried out on a hot plate for 3-4 h at 120ºC into the Teflon® containers using a mixture of nitric (BDH Aristar) and perchloric acids (BDH Aristar) with a 2:1 ratio respectively. The residual acid solutions were diluted with Milli-Q deionized. Finally acid solution was transferred to high density polyethylene (HDPE) bottles.

Copper (Cu), Cadmium (Cd) and lead (Pb) analysis was performed by atomic absorption spectrometry (GFAAS and FAAS- only Zn) and using a Varian SpectrAA 220 Zeeman and 220FS. For Cd and Pb determinations, a $NH_4H_2PO_4$ matrix modifier was applied. Standard addition method was used and, at least triplicate determination of each sample was carried out. Data quality control was provided by a separate comparative study of a standard reference material (BCR CRM 278 -mussel tissue). The agreement between the results for the reference biological material CRM certified values was satisfactory (table 1).

Table 1: Analysis of certified reference material (CRM 278 mussel tissue): certified values, found and recovery.

Metal	Certified (µg g^{-1} dry wt.)	Found (µg g^{-1} dry wt.)	Recovery %
Cu	24.73 ± 0.34	24.93 ± 1.14	100.8
Cd	0.911 ± 0.018	0.788 ± 0.06	86.50
Pb	5.234 ± 0.10	4.92 ± 0.23	94
Zn	217.47 ± 4.44	217.5 ± 11.52	100.0

2.3 Statistical analysis

Mean metal concentrations together with standard errors were calculated for overall registered data. To check up on the differences in the metal levels a Kruskal-Wallis test was previously used for each species. Non-parametric Mann-Whitney U-tests were then conducted to test the significance of the differences in the metal concentrations among islands. Associations between size, weight and Cd concentration were studied by means of non-parametric Spearman correlation coefficient. All analysis were performed using the package SPSS version 12 © software for Windows XP.

3 Results

The mean of total metal concentrations were different between both species of limpets and it decreased according to the sequence of Zn >> Cu > Pb > Cd and Zn >> Cu > Cd > Pb in *P. rustica* and *P. candei crenata* respectively.

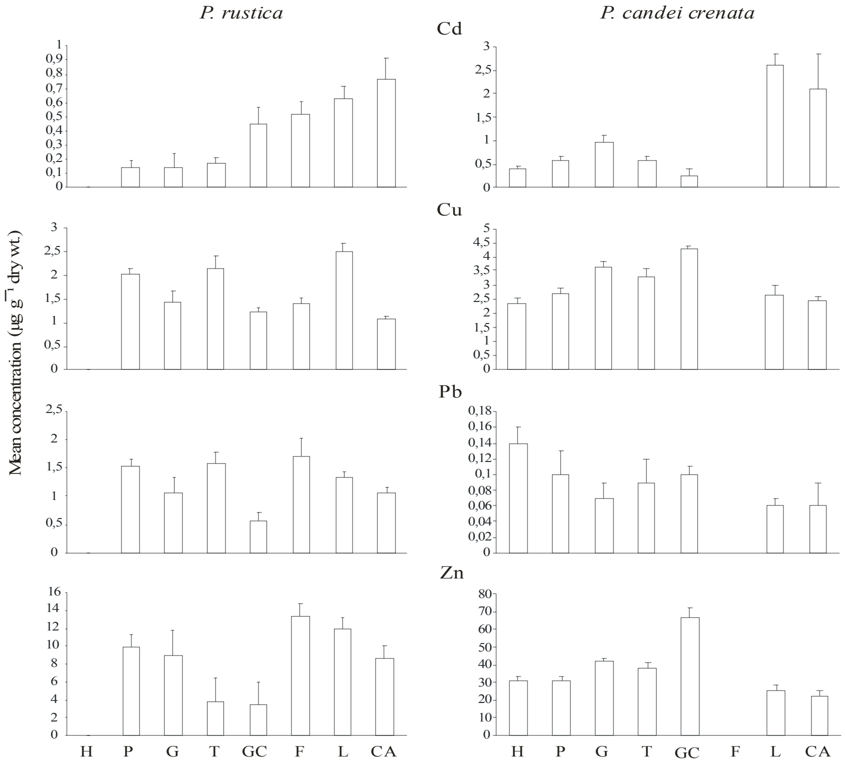

Figure 2: Levels of Cd, Cu, Pb and Zn (µg g^{-1} dry wt., mean ± S.E) in the studied gastropods from the Canary Islands (CA: Chinijo Archipelago; L: Lanzarote; F: Fuerteventura; GC: Gran Canaria; T: Tenerife; G: La Gomera; P: La Palma; H: El Hierro).

The highest mean values (µg.g^{-1} dry wt.) in *P. rustica* were 0.77 ± 0.14 (Cd; Chinijo Archipelago), 2.51 ± 0.17 (Cu; Lanzarote), 1.70 ± 0.33 and 13.38 ± 1.39 (Pb and Zn; Fuerteventura) (mean ± S.E.) (Table 2) (fig. 2). On the other hand, *P. candei crenata* showed: 2.60 ± 0.23 (Cd, Lanzarote), 4.27 ± 0.11 (Cu, Gran Canaria), 0.14 ± 0.02 (Pb, El Hierro) and 66.80 ± 5.53 (Zn, Gran Canaria) µg g^{-1} dry wt. (mean ± S.E.).

Only negative correlations between metal concentrations and body weight and size were observed in *P. rustica* (table 3). On the other hand, *P. candei crenata* showed negative correlations between both biometric parameters and the Pb, positive correlation between the Cd and size, whereas, it did not exhibit any correlation with Cu and Zn (Table 3). In both species of gastropods we obtained positive correlations between some metals (table 3).

Table 2: Ranges (min-max) of the heavy metals and the biometric parameters measured in *P. rustica* and *P. candei crenata* at different islands. Data: size (mm), weight (g) and metal concentrations (μg g^{-1}dry wt.) P.r: *Patella rustica*; P.c.c: *Patella candei crenata*.

		n	Size	Weight	Cd	Cu	Pb	Zn
Chinijo Archipelago	P. r	18	22-36	0.17-0.63	0.02-2.1	0.54-2.18	0.43-1.68	1.61-25.65
	P. c. c	8	39-48	0.26-0.69	0.85-5.39	1.98-3.23	0.02-0.24	15.92-43.54
Lanzarote	P. r	32	19-38	0.11-1.1	0.25-2.48	1.73-5.01	0.33-3.2	6.32-33.23
	P. c. c	8	39-47	0.21-0.55	1.74-3.45	2.16-5.01	0.05-0.10	14.93-42.71
Fuerteventura	P. r	10	18-33	0.11-0.45	0.25-1.22	0.79-1.9	0.81-3.4	8.87-21.59
	P. c. c	–	–	–	–	–	–	–
Gran Canaria	P. r	6	24-33	0.32-1.11	0.22-1.06	0.97-1.55	0.33-0.93	1.78-15.15
	P. c. c	3	31-39	0.13-0.34	0.12-9.16	4.09-4.48	0.09-0.10	61.51-72.54
Tenerife	P. r	12	21-30	0.1-0.39	0.05-0.55	0.1-0.39	0.57-3.4	0.95-2.35
	P. c. c	21	22-52	0.03-0.73	0.08-2.05	1.63-5.93	0.02-0.54	20.19-71.48
La Gomera	P. r	9	27-34	0.09-0.77	0.03-0.89	0.83-3.17	0.48-2.43	4.03-25.73
	P. c. c	32	29-53	0.05-0.78	0.21-3.87	1.70-6.73	0.01-0.55	24.74-68.70
La Palma	P. r	17	20-33	0.15-0.49	0.01-0.61	1.48-2.97	0.74-2.41	0.14-24.06
	P. c. c	20	28-44	0.15-0.57	0.23-1.86	1.45-4.52	0.02-0.47	15.94-52.24
El Hierro	P. r	–	–	–	–	–	–	–
	P. c. c	29	29-49	0.11-0.90	0.10-1.09	0.71-6.59	0.04-0.38	11.31-54.35

4 Discussion

The variability in metal concentrations seems to reflect the great differences (interspecific and intraspecific) in both species of gastropods. The mean levels of Cu, Cd and Zn were, in general, higher in *P. candei crenata* than in *P. rustica*, which had a higher mean concentration of Pb, consequence of different pattern in metal accumulation process. These species present some differences regarding to their vertical distributions (tidal height of habitat) in the intertidal zone of the Canary Islands [6], that can affect to their food supply (type of microalgae) and then to provoke differences in the metals uptake [13, 14]. These variations due to microhabitat can cause differences in metal concentrations.

Table 3: Significant Spearman correlations coefficients (r) and levels of significance (p). Lines indicate no significant relationship

	P. rustica		*P. candei crenata*	
	r	p	r	p
Size-Weight	0.689	(0.000)	0.746	(0.000)
Size-Cd	–		0.219	(0.02)
Size-Cu	– 0.202	(0.041)	–	
Size-Pb	– 0.682	(0.000)	– 0.251	(0.006)
Size-Zn	– 0.224	(0.025)	–	
Weight-Cd	–		–	
Weight-Cu	– 0.313	(0.001)	–	
Weight-Pb	– 1	(0.000)	– 0.443	(0.000)
Weight-Zn	– 0.435	(0.000)	–	
Cd-Cu	–		–	
Cd-Pb	–		–	
Cd-Zn	–		–	
Cu-Pb	0.338	(0.001)	–	
Cu-Zn	–		0.350	(0.000)
Pb-Zn	0.442	(0.000)	0.186	(0.048)

Metal concentration in an organism is greatly influenced by his size [13, 14]. According to van Roon [13], negative correlations are more commonly observed than positive ones [15]. This fact has been observed in our study. Likewise, the weight (well-fitted with the size) presented only negative correlations (e.g. Pb). For some metals, especially for Pb, Davies and Hatcher [16] found that metal concentrations were higher in the small individuals and in their mucus. They suggested that mucus could be a depuration route in *Patella vulgata*. Besides, mucus production was related with the pedal area. It is supposed that small individuals or same species of limpets (like *P. rustica*) have less mucus production provoking less excretion of metals (e.g. Pb). In contrast to this, we observed that *P. candei crenata*, which present higher sizes and therefore larger pedal areas had low concentrations of Pb (table 2).

Cd concentrations variations could be related with a regional scale phenomenon. It evidenced a decreasing tendency between eastern (high concentrations) and western islands (low concentrations). It could be related with the up-welling processes that take place at northwest African coast [17]. As a result of this oceanographic event, near shore waters of the eastern islands (Chinijo Archipelago, Lanzarote, Fuerteventura and Gran Canaria) are under regular influenced of these up-welled cold-water inputs [18]. Segovia-Zavala *et al* [20] registered at the coastal up-welling area (California-Mexico border) that the maximum concentrations (0.14-0.166 nM) of Cd were found inshore, whereas the lowest concentrations (0.03-0.058 nM) were registered offshore (50 km); this pattern was also observed by Collado [21] along parallel 29º, at the north of the Canary Islands. Thus, this increase in local bioavailability of Cd, whether dissolved or in the diet, can cause an increase in the uptake rate of Cd into the body [21–24].

Interactions between metal are known [25], and these must be take into account in biota studies. In our analyses only positive correlations between metals were found. Cu and Zn, essential metals, were related between them and with Pb (a non-essential metal). Heavy metal contaminations of the considered species were, in ranges, comparable to those reported in other published works (table 4). We have found strong significance differences between islands, but we have not observed that the highest metal concentrations were present in the more inhabited islands (Tenerife and Gran Canaria).

Table 4: Ranges of metal concentration for Patella *spp*. obtained in this study and data previously provide from other geographical areas.

Species	sites	Ranges of concentration (μg g^{-1} dry wt.) Cd	Cu	Pb	Zn	references
Patella rustica	Canary Islands	0.01-2.48	0.54-5.01	0.33-3.4	0.14-33.23	This work
Patella candei crenata	Canary Islands	0.08-5.39	0.71-6.59	0.01-0.54	11.31-72.54	This work
Patella spp.	Tenerife, Canary Islands	0.09-7.54	0.10-15.2	0.50-6.36	0.33-48	[9]
Patella spp.	Gran Canaria, Canary Islands	0.01-1.07	0.7-13.56	0.05-1.67	–	[10]
Patella caerulea	Sicily, Mediterranean	1.7-11.8	0.47-3.79	0.06-2.18	2.2-19.1	[14]
Patella lusitanica	Sicily, Mediterranean	2-6.6	1.42-3.90	0.10-1.02	5.8-21.3	[14]
Patella aspera	Portugal, clean site	3.5-9.1	3.5-9.2	< 0.1	36.1-114.2	[25]
Patella aspera	Portugal, contaminated site	1-2.6	4.2-15.2	< 0.1	73.4-172.0	[25]

The use of indigenous limpets has allowed us to test the bioavailability of metal loads, providing a new baseline of data. Thus, *Patella* species have a considerable potential as cosmopolitan biomonitors of heavy metals in the Canary Islands, especially, *Patella rustica.*

Even so, further research is needed to clarify the actual accumulation patterns of these species, considering other parameters such as season variations, sex, reproductive stage, etc or to include new possible species as biomonitors (*Patella aspera*, *Osilinus* spp., etc).

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