

## Gastropod molluscs as indicators of the cadmium natural inputs in the Canarian Archipelago (Eastern Atlantic Ocean)

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### Abstract

Nowadays, gastropod molluscs are being utilized more and more as bioindicator organisms. Similarly, harmful metals on human health such as cadmium have been widely studied. The Canarian Archipelago (specifically the eastern islands) is constantly bathed by the African coastal upwelling, provoking oceanographic and biological differences between the islands. This process could assume an increase in the Cd concentration in their coastal waters and in the biota. Thus, in order to assess this fact, we measured cadmium concentrations in the soft parts of two species of limpets (*Patella rustica* and *Patella candei crenata*) and in a topshell snail (*Osilinus atrata*). Metal determination was performed using atomic absorption spectrometry (AAS). We found significant differences for metal concentrations between the eastern islands and the western islands for all species. *P. rustica*, *P. c. crenata* and *O. atrata* presented values ranging from 7.71, 2.11 and 7.56  $\mu\text{g g}^{-1}$  dry wt. (eastern islands) to 1.38, 0.4 and 1.08  $\mu\text{g g}^{-1}$  dry wt. (western islands) respectively. Therefore, we concluded that limpets and topshell snails seem to be suitable indicators of the cadmium concentrations in the coastal waters of the Canary Islands.

**Keywords:** *Canary Islands, heavy metals, cadmium, gastropod molluscs, Patella rustica, Patella candei crenata, Osilinus atrata.*



## 1 Introduction

Cadmium (i.e. Cd) is a non-essential metal for life and it can be toxic even at low concentrations. Its harmful effects on humans have made it one of the most studied metals. In the oceans, its concentration is related with the nutrients phosphate and nitrate concentrations [1]. Besides, all of them present higher concentrations in deep waters than in the ocean surface. In contrast to this, Cd can be found at higher concentrations in the surface waters when a coastal upwelling process has happened [1]. Furthermore, any increase in the Cd load in the nearshore waters can have an immediate effect on the levels of this metal in the biota [2]. In this sense, organisms can be reflecting into their bodies an increase in the local bioavailability of the metal due to natural and human inputs. Thus, molluscs represent one of the best groups of the animal kingdom to be utilized as bioindicators [3].

The Canary Islands lie in a transition zone between the oligotrophic open ocean and the northwest African upwelling region (so-called Northwest African Coastal Transition Zone [NACTZ]) [4]. Quasi-permanent filaments of these waters reach the eastern islands (Chinijo Archipelago, Lanzarote, Fuerteventura and Gran Canaria) of the Canarian Archipelago. As a result, eastern and western islands present differences in their oceanographic characteristics [5, 6]. In addition, in the rocky coasts of the Canary Islands exist several species of gastropods molluscs, like limpets and topshell snails. These could be utilized as bioindicators for metal entrances in the coastal environments, such as what has been done in other geographical areas (e.g., [7–11]). We must take into account that food is one of the major entrance of Cd into humans [2]. In our case, the limpets and topshell snails are widely harvested and consumed by local people throughout the Canarian Archipelago [12, 13]. This could suppose a harmful metal transfer that, until now, has not been valued in the realized medical studies [14].

Therefore, our objective was to determine if the limpets (*Patella rustica* and *Patella candei crenata*) and the topshell-snail (*Osilinus atrata*) can be used as indicators for the natural inputs of Cd in the Canarian Archipelago.

## 2 Material and methods

### 2.1 Study area and sampling

Our study was carried out in the Canary Islands (13–19° W, 27–30° N), in March 2003 (Figure 1). Organisms were randomly handpicked from each location (three per islands) according to their availability. Specimens of *Patella candei crenata* were not found in Fuerteventura Islands and *Patella rustica* was not found in El Hierro Islands. Samples (*P. rustica*, n=104, *P. candei crenata*, n=121, *Osilinus atrata*, n=112) were placed in polypropylene bags and transported to the laboratory where each individual was measured (total length), rinsed with deionized water (Mili-Q, Millipore, 18 MΩcm), and frozen in new bags until the digestion processed [15, 16].



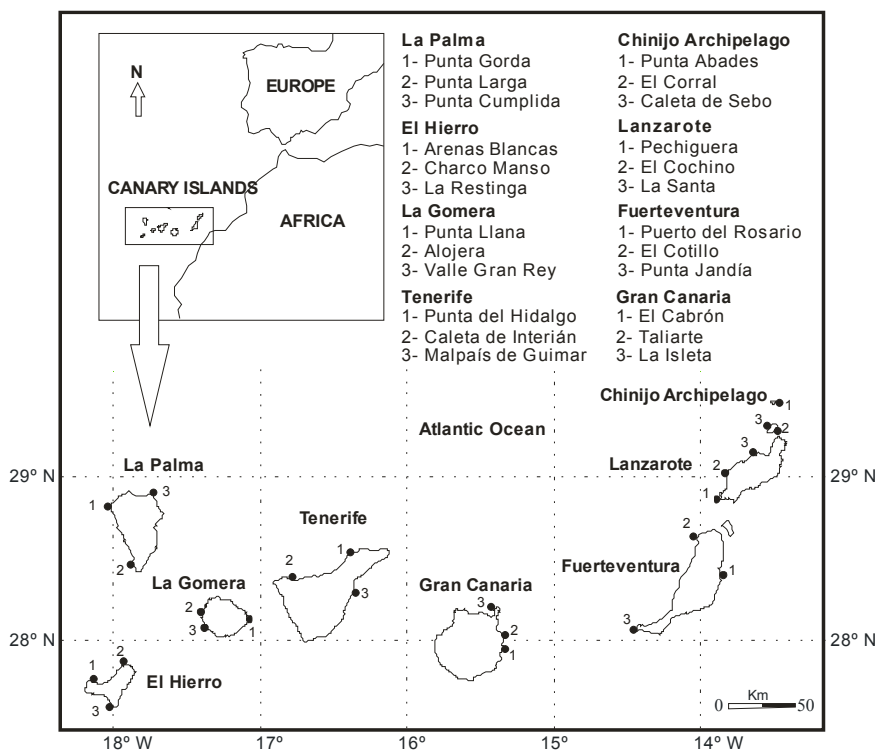


Figure 1: Map of the study area which shows the locations where the mollusc samples were recollected.

## 2.2 Analysis

Metal determination requires careful sample handling, storage and analysis. Therefore, all plastic materials were previously treated with an acid cleaning. It consisted in successive rinsing with diluted nitric (0.3 M) and hydrochloric (0.1 M) acid solutions for 72 hours, and washing with water of analytic quality (Millipore Milli-Q system, 18 MΩ.cm) [15, 16].

We proceed to extract the soft tissues from their shells and to clean them to remove the salts and impurities. To obtain the dried weight, the samples were dried with a IR lamp until constant weight (48 h). Then, the samples digestion was carried out on a hot plate for 3-4 h at 120°C, using a mixture of nitric and perchloric acids with a 2:1 ratio (4ml:2ml). Finally, the residual acids solutions were diluted up to 100 ml with Mili-Q deionized water.

The metal concentrations analysis was calculated using atomic absorption spectrometry (AAS). In particular, the Cd concentration was performed by graphite furnace atomic absorption spectrometry (GFAAS). Besides, for its determination a matrix modifier was used (NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>). Data quality control was provided by a separate comparative study of a standard reference material (BCR

CRM 278-mussel tissue). This was satisfactory showing a recovery of 86.50%. Each sample, blanks and the certified reference material were carried out in triplicate.

### 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 the 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 total concentrations of Cd for *Patella rustica*, *Patella candei crenata* and *Osilinus atrata* were  $3.61 \pm 0.45$ ,  $0.71 \pm 0.10$  and  $2.55 \pm 0.45 \mu\text{g g}^{-1}$  dry wt. (mean  $\pm$  S.E) respectively. Similarly, the mean total size and weight from overall individuals analysed were  $27.37 \pm 0.40$ ,  $38.08 \pm 0.58$  and  $16.48 \pm 0.35$  (mm  $\pm$  S.E) for the size, whereas the weight were as follows  $0.32 \pm 0.02$ ,  $0.33 \pm 0.02$  and  $0.11 \pm 0.01$  (g  $\pm$  S.E) for *P. rustica*, *P. candei crenata* and *O. atrata* respectively. With respect to the islands, the maximum concentrations were found in the Chinijo Archipelago ( $7.71 \pm 1.39 \mu\text{g g}^{-1}$  dry wt., *P. rustica*;  $7.56 \pm 0.83 \mu\text{g g}^{-1}$  dry wt., *O. atrata*) and Lanzarote ( $2.11 \pm 0.73 \mu\text{g g}^{-1}$  dry wt., *P. c. crenata*) (Figure 2). We also observed a high value for *O. atrata* ( $4.34 \pm 0.83 \mu\text{g g}^{-1}$  dry wt.) in La Palma. In contrast to this, the minimum values were registered in La Palma for the limpet *P. rustica* ( $1.38 \pm 0.48 \mu\text{g g}^{-1}$  dry wt.) and in Gran Canaria for *P. c. crenata* and *O. atrata* ( $0.25 \pm 0.16$  and  $0.58 \pm 0.07 \mu\text{g g}^{-1}$  dry wt. respectively) (Figure 2).

For each species, we have detected significance differences ( $p < 0.01$ ) between the studied islands (Kruskal-Wallis test). The results obtained with the Mann-Whitney U-tests are reported in the table 1. The Cd showed a tendency from eastern (high concentrations) to western islands (low concentrations) with great significant differences (Table 1).

Only positive correlations were found between the size and the Cd concentrations for *Patella candei crenata* ( $r = 0.219$ ,  $p = 0.02$ ) and *Osilinus atrata* ( $r = 0.292$ ,  $p < 0.01$ ). However, for *Patella rustica* we did not registered any relationship between this biometric parameter and the Cd concentrations. Strong correlations were detected between the size and weight of the three species ( $0.689$ , *P. rustica*;  $0.746$ , *P. c. crenata* and  $0.971$ , *O. atrata*;  $p < 0.0001$ ). On the other hand, for all species the weight did not show any relationship with the measured Cd concentrations.



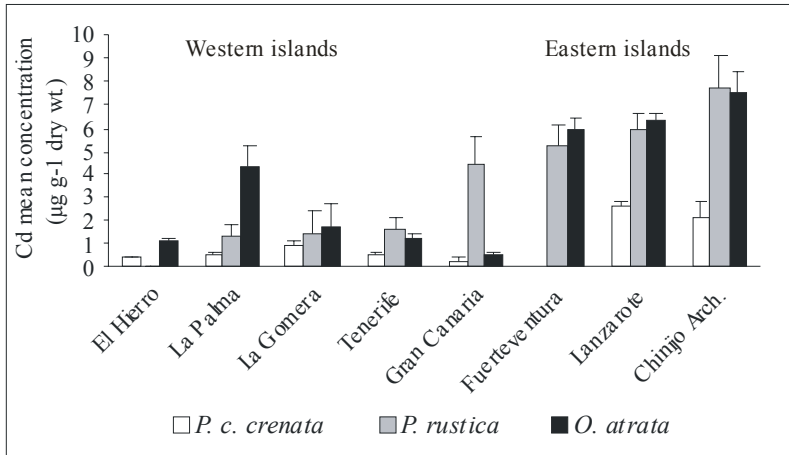


Figure 2: Mean concentration of Cd ( $\mu\text{g g}^{-1}$  dry wt.  $\pm$  S.E) in the three studied gastropods throughout the Canarian Archipelago.

Table 1: Differences on metal concentration obtained for the different islands using the U-Mann Whitney tests.

Level of significance			
	$0.01 < p < 0.05$	$0.001 < p < 0.01$	$p \leq 0.001$
<i>P. rustica</i>	CA-F, CA-GC, F-G, GC-P	CA-G, GC-T	CA-T, CA-P, L-T, L-G, L-P, F-T, F-P
<i>P. c. crenata</i>	CA-GC, CA-G, T-G	CA-T, G-P	CA-P, CA-H, L-T, L-G, L-P, L-H, G-H
<i>O. atrata</i>			CA-GC, CA-T, CA-G, CA-H, L-GC, L-T, L-G, L-H, F-GC, F-T, F-G, F-H, GC-T, GC-G, GC-P, T-P, G-P, P-H

CA: Chinijo Archipelago, L: Lanzarote, F: Fuerteventura, GC: Gran Canaria, T: Tenerife, G: La Gomera, P: La Palma; H: El Hierro



## 4 Discussion

Our results have shown a clear tendency of the Cd concentrations from the eastern to western islands. Besides, this accumulation pattern at regional scale is constant for the three studied species of gastropod molluscs. We have not found a clear reason to explain the relatively high peak of Cd that was found for *Osilinus atrata* in La Palma. Differences between the oceanographic characteristics (e.g., T<sup>a</sup>, salinity, nutrients concentration, etc) in the Canarian Archipelago have been shown in multitude of works (e.g., [5, 6, 17]). This variation can imply changes at biological level of the populations (e.g., abundance) (e.g., [18, 19]). Moreover, our work show that the upwelling process which takes place in the northwest coast of Africa, provoke physiological alterations under a chemical point of view. In this sense, there are evidences that associate Cd concentrations with these kinds of events, such as what have occurred in Baja California and the Moroccan coast for two different species of mussels [20, 21].

On the other hand, Cd concentrations obtained in the eastern islands were similar for *P. rustica* and *O. atrata*, whereas *P. c. crenata* showed lower concentrations. It is known that the gastropods related species may exhibit distinct accumulation strategies for heavy metals [22–25]. The studied gastropods present some differences regarding to their vertical distributions (tidal height of habitat) in the intertidal zone of the Canary Islands [12]. It could affect to their food supply (type of microalgae) and consequently provoke differences in the metals uptake [11, 26, 27]. Thus, an enhance in local bioavailability of Cd, whether dissolved or in the diet, can cause an increase in the uptake rate of that metal into the body [24, 28]. In consequences, Cd variations into their bodies seem to be due to external facts more than to the individuals' characteristics.

Finally, biometrics parameters showed light or none relationships with the Cd levels found in the studied molluscs. Despite to this, the correlations were always positive as it was observed in the Mediterranean area with similar gastropod species [11]. This means that the largest individuals presented higher lightly Cd concentrations than those found in the smaller individuals. Even though, we need more information to clarify the actual accumulation patterns of these species, taking into account other parameters such as season variations, sex, reproductive stage, etc and to include new possible species as bioindicators.

## 5 Conclusion

We concluded that limpets (*Patella rustica* and *Patella candei crenata*) and topshell snails (*Osilinus atrata*) could be suitable organisms to be used as indicators in the Canary Islands. They clearly reflect the Cd natural inputs in the Canarian Archipelago coming of the African up-welling zone.



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