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ORIGINAL ARTICLE

Study of metals concentration levels in *Patella piperata* throughout the Canary Islands, Spain

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Abstract In order to assess the extent of metal contamination at rocky shores of the Canarian Archipelago, metal concentrations have been measured in *Patella piperata* (Gould, 1846), using the standard atomic absorption spectrophotometer technique. Ranges of elements concentrations measured (in μg g^{-1}) found in the biota were: Cd (0.36 ± 0.26 μg g^{-1} dry wt.), Cu (2.05 ± 0.91 dry wt.), Pb (1.57 ± 1.14 μg g^{-1}dry wt.) and Zn (10.37 ± 4.60 μg g^{-1} dry wt.). Variation in metal concentrations in *Patella*, was tested by using non-parametric statistical methods. Cd content had a maximum in the Archipelago Chinijo, northward of Lanzarote Island. The metal concentrations recorded at the clean stations may be considered carefully if they are used like background levels.

Keywords Heavy metals · Biomonitor · *Patella piperata* · Canary Islands

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1. Introduction

Gastropods molluscs are frequently used as sentinel organisms to biomonitor the metal levels in coastal environments (Goldberg, 1975; Rainbow and Phillips, 1993; Brown and Depledge, 1998). These species accumulate metals in their tissues responding essentially to the fraction present in the environment, which is of direct ecotoxicological relevance (Rainbow, 1995; Rainbow and Furness, 1990). These organisms have the advantage over other measurements because they concentrate the portion of the metals biologically available, which is usually of interest when assessments are being made of metal contamination (Luoma, 1983; Phillips, 1990; Dallinger and Rainbow, 1993). Furthermore when it is known toxicity is dependent on the bioavailable exposure concentration (Ilyin *et al.*, 2003). Filtering organisms are usually used, although other indigenous species are now being more frequently utilized in rocky coasts in recent years (Prosi, 1983; Bryan *et al.*, 1985; Phillips, 1985; Langston, 1990; Campanella *et al.*, 2001; Conti *et al.*, 2003; Cravo *et al.*, 2004). The lack of species like mussels has focused our attention on other possible biomonitors such as the *Patella* spp. We selected *Patella piperata*, which is an important microphagous grazer on intertidal rocky shores. Additionally, *Patella piperata* is largely harvested by local people throughout the Canarian Archipelago (Moro *et al.*, 2000) and it could be a route of entrance of contaminants that should be measure to prevent health problems. The wide distribution of the *Patella piperata* along the Canarian coasts (Navarro *et al.*, 2004) makes it possible to compare regional differences in metal concentration.

The Canary Islands have seen a spectacular increase of population and consequently urban and industrial areas. Rapid growth of urban centres, together with increasing investments in agriculture and industry wastes could be resulting in rapid increase in the pollution and pollutants. Waste waters could be discharging heavy metals in the coastal areas. Metals, when in excess, are toxic to organisms and persistent in the aquatic environment (Bryan, 1984; Nriagu, 1990). Until now no study has valued the concentrations of such contaminants in organisms all over the Canary Islands. Thus, our objective was to determine metal concentrations (Cd, Cu, Pb and Zn) in the soft parts of the limpet species *Patella piperata*, which allows us to establish a preliminary metal baseline level in these organisms. At the same time, we were searching to see if these elements present any distribution pattern throughout the study area. So far very little information about background concentrations has been available. Finally, we expected to evaluate if the obtained concentrations could have a supposed risk for human.

Area of investigation

The study was based in the Canarian Archipelago, with a total of 24 sites (three per islands) distributed throughout the coast of the Canary Islands (including the small islets called the Chinijo Archipelago, northward of Lanzarote island), during March and April 2003, Spain (Fig. 1).

Methodology

Sampling and sample treatment

The molluscs were harvested by hand with a stainless steel knife from the intertidal zone at minimal low tide levels. Animals were placed in metal-free plastic bags and transported to the laboratory. Before the digestion of the individuals, they were washed and frozen in polypropylene bags until processing (Moody and Lindstrom, 1977).

Biological samples were digested using a well-known digestion technique (Bryan *et al.*, 1985). The soft tissues of the molluscs were carefully extracted with a plastic spatula. To obtain dry weights the limpets were dried with an IR-light lamp. Then, to digest the soft tissues, they were placed in a Teflon container with a mixture of acids (4 ml HNO_3: 2 ml $HClO_4$) and placed on a hot plate for 3 hrs at 120°C. After digestion, the samples were transferred to a beaker and diluted up to 100 ml with Mili-Q deionized water.

Trace element impurities were removed from glassware, high-density polyethylene bottles, and Teflonware by through cleaning, acid washing (72 hrs, HNO_3 3M: 72 hrs, HCl 0.1 M) and rising with Mili-Q deionized water (72 hrs).

Metal analysis

Analysis for Cd, Cu and Pb was performed by graphite furnace atomic absorption spectrometry (GFAAS) using a Varian Spectra AA250 atomic absorption spectrophotometer via the standard addition procedure. Zn was determined by flame atomic absorption spectrometry (FAAS). For Cd and Pb determination a $NH_4H_2PO_4$ matrix modifier was applied. Detection limits were calculated according to mean blank plus 3 standard deviation of the blank values. These values (μg g^{-1} dry wt) were: 1.63, 3.68, 7.65 and 0.15 μg g^{-1} dry wt, respectively (Currie, 1997). Data quality control was achieved using a standard reference material (BCR CRM 278 - mussel tissue). Recovered results obtained (in *percentage*) were: Cu (97%), Pb (91%), Zn (111%) and Cd (112%).

Statistics

Descriptive data analysis (mean and standard deviation) was carried out to determine relationships among different metals. Variation in metal concentrations in Patella harvested from different sites was tested by using non-parametric statistical methods. Inherent variability existing between locations in the same island was not considered due to the fact that the aim was to obtain only the variability between islands. Thus, data was pooled per islands to test our objective. Kruskal-Wallis test was previously used to check on the differences in the metal levels registered. Non-parametric Mann-Whitney U-tests were then conducted to test the significance of the differences in the metal concentrations between islands. The level of significance was set at $p < 0.05$. The differences in average levels were

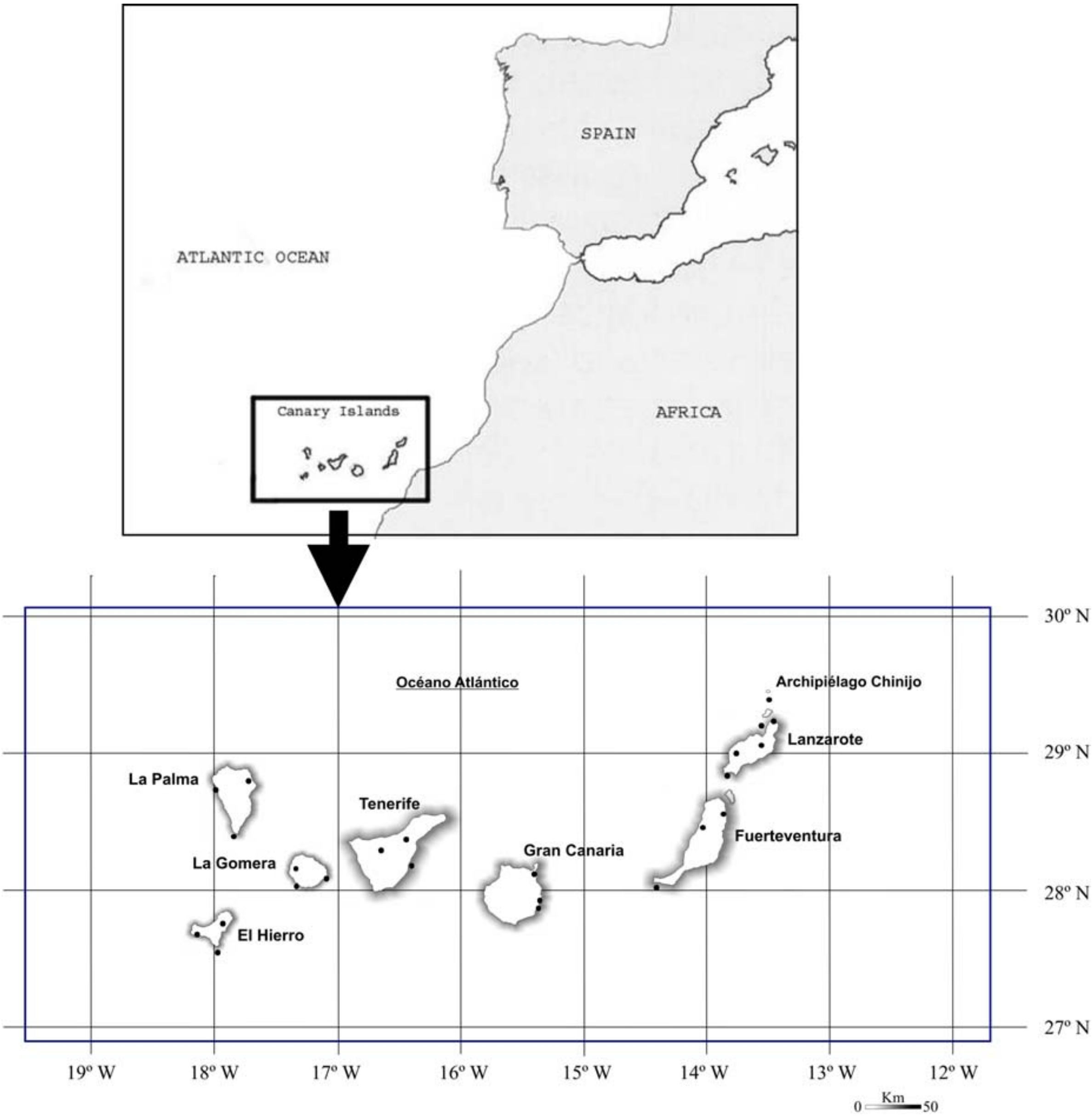

Fig. 1 Collecting localities in the Canary Islands Archipelago, Spain.

assessed using the SPSS version 11.5 © Software for Windows XP.

Results and discussion

Mean concentrations of Cd, Cu, Pb and Zn in *Patella piperata* collected in our study are shown in Table 1. The individual concentrations of the metals studied ranged as follows: $10.37 \pm 4.60\ \mu g\ g^{-1}$ (Zn), $2.05 \pm 0.91\ \mu g\ g^{-1}$ (Cu), $0.36 \pm 0.26\ \mu g\ g^{-1}$ (Cd) and $1.57 \pm 1.14\ \mu g\ g^{-1}$ (Pb). The metal concentrations in the soft tissues decreased according to the sequence of $Zn \gg Cu > Pb > Cd$. Data that compare metal concentrations in the soft tissues of *Patella piperata* are sparse and there are very few references about similar works (Díaz *et al.*, 1992; Collado *et al.*, 1999).

Kruskal-Wallis test detected significance differences ($p < 0.01$) among the islands for the four studied metal concentrations at the different stations. As seen

Table 1 Ranges of metal concentration for Patella piperata

Islands	*n*	Cd	Cu	Pb	Zn
Chinijo Archipelago	18	0.662 ± 0.209	1.104 ± 0.220	1.136 ± 0.430	8.984 ± 3.016
Lanzarote	32	0.520 ± 0.203	2.787 ± 0.868	1.448 ± 0.633	12.052 ± 3.40
Fuerteventura	10	0.289 ± 0.110	1.409 ± 0.304	2.094 ± 1.115	13.916 ± 4.420
Gran Canaria	6	0.271 ± 0.078	1.243 ± 0.226	0.629 ± 0.325	3.213 ± 1.323
Tenerife	12	0.149 ± 0.075	2.673 ± 0.643	1.820 ± 0.729	6.082 ± 3.402
La Gomera	9	0.039 ± 0.011	1.281 ± 0.321	2.489 ± 3.096	7.119 ± 1.947
La Palma	17	0.147 ± 0.119	2.223 ± 0.348	1.670 ± 0.543	13.144 ± 4.702
Total	104	0.363 ± 0.265	2.051 ± 0.910	1.578 ± 1.145	10.377 ± 4.608
		(0.023–0.941)	(0.6–5.01)	(0.332–10.213)	(1.61–24.06)

Data: mean ± S.D (μg g^{-1}); Total range (min-max).

in Table 2, where the results obtained with the Mann-Whitney U-tests are shown, data clearly demonstrate that there are some significant ($p < 0.05$) spatial variations of the four element concentrations.

Cd contents in Patella shows a different layout. Cd element ranged from 0.03 to 0.66 μg g^{-1} dry wt. The island of La Gomera was characterized by Cd tissue concentrations slightly lower than those found in the other places (Table 1). Opposed to this, Cd concentrations in the soft tissues at Chinijo and Lanzarote were significantly higher than those obtained at the other sampling sites; presumably much more contaminated (Table 1). Similarly, the expected clean shores at north of Lanzarote are high in Cd. It seems interesting to emphasize that significant differences between the neighbouring islands do not exist, although Tenerife and Gran Canaria do not satisfy this condition (Table 2). Moreover Gran Canaria does not present significant differences with Lanzarote and La Gomera (Table 2). Cd showed a tendency to decrease with distance to the African coast. Oceanographic conditions may thus vary on scales that generate significant variability in metals bioavailability at the bottom of the trophic-net, and through upward-flowing food chain effects. It is known cadmium concentration in the ocean is related to the nutrients, and both present higher concentrations in deep waters than in the ocean surface (Bruland, 1983). These can be found at higher concentrations in the surface waters when up-welling processes has happened (e.g. at northwest African coast). High Cd levels, on the north coast of the Archipelago, could be explained by a natural input of dissolved Cd probably originated by the plumes coming from the saharian upwelling zone, a very intense phenomenon in the northeastern Atlantic upwelling system due to the location of the Canarian Archipelago along the Northwest African Coastal Transition Zone (NACTZ) (Barton *et al.*, 1998). Such Cd enrichments have already

Table 2 Differences obtained on metal for the different islands using the U-MannWhitney tests

Cd		Cu		Pb		Zn
CA-F*	F-T***	CA-L***	F-T**	CA-GC**	T-G*	CA-F**
CA-GC*	F-G*	CA-F**	F-P***	CA-T**	G-P*	CA-P*
CA-T***	F-P***	CA-T***	GC-T*	CA-P**		L-GC**
CA-G**	GC-T**	CA-P***	GC-P***	L-GC***		L-T*
CA-P***	GC-P*	L-F***	T-P*	F-GC**		F-GC*
L-T***		L-GC***		F-G*		F-T*
L-G***		L-G***		GC-T***		GC-P*
L-P***		L-P**		GC-P***		T-P*

Level of significance: *:$0.01 < p < 0.05$; **:$0.001 < p \leq 0.01$; ***:$p \leq 0.001$.
CA: Chinijo Archipelago, L: Lanzarote, F: Fuerteventura, GC: Gran Canaria, T: Tenerife, G: La Gomera, P: La Palma.

been reported in molluscs in study areas of the same type on the Moroccan and Mauritanian coast and the California coast (Segovia-Zavala *et al.*, 1998; Banaoui *et al.*, 2004). In addition, we presume that limpets in these areas graze on a larger variety of epilithic algal resources and, consequently, have a different pattern of accumulation. At the same time, high values of atmospheric aerosols have been found in Gran Canaria and Tenerife (0.3–0.6 ng/m3) for cadmium which could have a decisive influence in those high values (Gelado M, personal communication). This fact may indicate atmospheric deposition of heavy metal aerosols in the study area (e.g., industrial activities, waste incineration, heating plants, crude oil processing and dust entries), may contribute to the bioaccumulation capacities of this species for the element. It seems difficult to explain in our case the higher bioavailability of cadmium associated to lithological differences as it has been found in other places (Bebianno *et al.*, 2003). It is important to note that several studies have indicated that the shell tends to accumulate almost as much or even more Cd in the soft tissue of molluscs, thereby reducing the amount available for internal uptake and elevating the threshold concentration (Wong, 1987). No data is available to corroborate it in our study.

High Cu concentrations were found in Lanzarote (2.78 μg g^{-1}), Tenerife (2.67 μg g^{-1}) and La Palma (2.22 μg g^{-1}) whereas Chinijo was the island with the lowest concentration value. The levels ranged from 1.10–2.78 μg g^{-1}. There are no significant differences between Lanzarote and Tenerife, although differences exists between Lanzarote and La Palma. The Cu concentration was quite different among Chinijo, Fuerteventura, La Gomera and Gran Canaria (Table 1). On the other hand, in Lanzarote, Cu was higher than in Tenerife, La Palma, and Fuerteventura (Table 2). In Gran Canaria, the mean value obtained for Cu was 1.24 μg g^{-1} which is somewhat lower than that reported by Collado (1999). Concentration found in specimins collected in Tenerife were also lower compared with those found by Díaz (1992). The high Cu concentrations does not display a clear relation either with limpets collected near wastewater inputs or with areas directly influenced by high ship traffic.

It is interesting to highlight that in the present study, the higher Pb concentrations in limpets (*Patella piperata*) were found in La Gomera and Fuerteventura, ranging from 0.63 to 2.48 μg g^{-1} dry wt all around the islands (Table 1). These values exceed the concentrations (total mean 1.57 μg g^{-1} dry wt) measured in *Patella* spp. in Díaz (1992) (mean: 1.19 μg g^{-1} dry wt). Comparing to this previous study, Pb concentrations seems to have increased in Tenerife since 1992, but the limpets were not collected in the same locations and any conclusion in this way could be wrong. It is not clear again from the results that Pb concentrations remain high at all stations, especially those in the vicinity of an urban area.

It must be kept in mind that, in the studied organism the actual uptake mechanism is probably rather complicated, because the exposure to trace elements is not limited to soluble metals in the aquatic medium. *Patella piperata* is herbivorous, though it can be supposed that the metals accumulated in the algae on which they graze affect their overall uptake.

The mean Zn concentration in the tissues was much greater than the levels of the other metals. Zn concentration also had a very high standard deviation compared to the others. The values did not fluctuate too much although they ranged from 3.21 to 13.91 μg g^{-1}. In all Patella species, there is a general tendency for Zn to be preferentially incorporated into the soft tissues (Cravo *et al.*, 2004). Variations in heavy metal concentrations are generally linked to particular local and temporal events such as outflows or industrial activities (Paek *et al.*, 1999). In relation to Zn, almost all of the samples showed concentrations lower than 14 μg g^{-1}. Despite the fact that Gran Canaria and Tenerife are the most populated and industrialized islands, they showed Zn mean values (dry weight) much lower than in other places. There was no reason found to explain this trend. The Canary Islands do not have a large industrial base and there are few secondary industry sources of heavy metals. In urbanised areas, treated sewage discharges may contribute small amounts of metals. Nevertheless, Zn concentrations were significantly different between sites ($p < 0.05$, Kruskal-Wallis test). It is observed that between the two islands where the zinc level is greater (Fuerteventura and La Palma), does not exist significant differences (Table 2). Fuerteventura and La Palma present significant differences with Chinijo, Gran Canaria y Tenerife, whereas Lanzarote only maintains those differences with Tenerife and Gran Canaria (Table 2).

In general, the concentrations of Cu, Pb and Zn in Patella are comparable to those reported by other authors in other places or coastal areas (Ramelow, 1985; Berrows, 1991; Miramand and Bentley, 1992;

Kondopoulos *et al.*, 2003; Cravo, 2004; Storelli, 2005). Although metal concentrations in the organisms were of the same order of magnitude, the concentrations generally fall in the range of the lowest values available in the literature for Cd, Cu and Pb. The comparative values cited by Miramand (2003), Cu concentrations in *Patella* from Favignana coast ranged from 1.2 to 2.4 μg g^{-1} dry wt while in samples from the southwestern part of England, high values reaching 42.3–45.9 μg g^{-1} dry wt were registered. Our Zn levels were considerably lower than those determined in the southwestern of England, Ireland and Norway (Miramand, 2003). In *Patella vulgate*, Goury (English Channel), near the outlet of a nuclear fuel reprocessing plant, the content of Cd reached 5.25 μg g^{-1} dry wt, was by far the highest value compared with ours. Considerably lower than ours were the values found in the Ionian Sea samples for Pb. In disagreement with our results, Storelli (2005) reported for Pb concentrations range from 0.11 to 0.54. μg g^{-1} dry wt. Thus, a comparison with values detected in specimens of the same species caught in other sea zones reveal that, our observations show that this area is for heavy metals weakly influence by anthropogenic activities. Finally, numerous studies conducted with *Patella spp*. have demonstrated that different requirements for metals and different metabolic and growth rates may explain same of these differences. Our current knowledge of the physiology of these gastropods is limited thus further interpretations cannot be made.

Conclusion

Biomonitors can be used to establish geographical and/or temporal variations in the bioavailabilities of heavy metals in the marine environment, offering time-integrated measures of those portions of the total ambient metal load. The metal levels of the considered species were, in ranges, comparable to those reported in the works published about coastal areas. *Patella piperata* has a considerable potential as cosmopolitan biomonitor of trace metals in the Canary Islands, since it shows different accumulation patterns per island, as well as being available in every season all over the rocky shores and being easy to sample and identify. General trends of bioaccumulation are hard to discern. The publication of this data could provide a new baseline of data, by which future local changes could be assessed. This first approach needs inexorably a future research on large spatial and temporal scales to asses the origin of the differences in metal concentrations found in the soft tissue of *P. piperata* species.

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