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Assessment of Heavy Metal Contamination of Seawater and Marine Limpet, *Patella vulgata* L., from Northwest Spain

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ABSTRACT

In the present work, both seawater and limpets (*Patella vulgata* L.) were collected from different sampling points in the Vigo estuary area (Galicia, NW Spain) and their heavy metal concentrations (Zn, Cd, Pb, Cu) were determined by means of a voltammetric technique. The results showed a direct relationship between the heavy metal levels in seawater and the content in biological samples, especially those obtained from limpet soft tissues, directly related to the increasing importance of anthropogenic environmental contamination. Heavy metal concentrations were usually higher in soft tissues than in shell, with a maximum difference corresponding to Cd and Zn (average of 93.6 and 86 folds, respectively). As expected, heavy metal content was higher in animal samples situated in inner part of the estuary zone, except for cadmium content in soft tissues, where a maximum of 5.62 ppm was quantified in limpet soft tissues collected next to the open sea area. Statistical analysis allowed the establishment of a clear relationship

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between Zn and Cd content in both shells and soft tissues from limpet, and especially between Zn and Cu in soft tissues ($p < 0.001$).

Key Words: Contamination; Heavy metals; Seawater; Limpet.

INTRODUCTION

Metals are commonly found in the environment all around the world, their presence being due to natural occurrence or as a result of anthropogenic activities.[1] Industrial activity has led to very high heavy metal concentrations on the environment, which are in general 100–1000 fold higher than those in the Earth's crust, and locally, living organisms can be exposed to even higher levels.[2] Along these lines, aquatic ecosystems and organisms are directly exposed to a great variety of these metals whose chemical forms and concentrations are governed by different processes, whether natural or not. These chemical agents are both essential elements required to support biological activities and nonessential metals with an unknown biological function, the latter being toxic to living organisms when subject to high concentrations.[3] Their negative effect is evident, not only by decreasing aquatic species diversity but by exposing human beings to these xenobiotics directly through the food chain, with a potential danger to human health.[4] Metals can enter and contaminate estuarine waters from feeder rivers and from direct discharges, and once there, they can be trapped and accumulated in sediments[5] or be directly captured by living organisms. An accurate evaluation of the degree of metal contamination at a given place requires an adequate estimation of natural levels of these elements in the biota and in the physical environment, too.[6]

The Ría of Vigo (Vigo estuary) is one of the largest and surely the best studied of several typical estuaries on the Northwest coast of Spain. The system has a relatively small river flowing into it and it also receives sewage and factory waste from the town of Vigo, which has over 400,000 inhabitants.[7] There are some important shipyards all around the estuary, mainly around the town of Vigo, which contribute to the general water contamination. On the other hand, this area possesses a high interest due to its potential as a main fish and shellfish producer for human consumption.

To determine heavy metal contamination, certain marine invertebrates are useful bioindicators, and sometimes the metal contamination levels in these animals are directly proportional to the available levels in the environment.[8] Transplanted or resident bivalves and other molluscs could provide us with a good indicator of temporally and spatially average concentrations of bioavailable contaminants in aquatic ecosystems.[9] Different limpet species have been used for aquatic biomonitoring because they are widely distributed (most rocky shores are inhabited by some type of limpet) and they concentrate heavy metals.[10]

The present study was conducted to investigate the heavy metal contents (Zn, Cd, Pb, Cu) in seawater and limpet collected from different sample locations, in order to establish, if possible, a good correlation between heavy metal contents and marine water contamination.

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MATERIAL AND METHODS

Sampling

Specimens of limpet, *Patella vulgata* L., and water samples were collected all along the Vigo estuarine area situated in Galicia (NW Spain), in different sampling stations, as shown in Fig. 1, and during the same journey (May 2002) sampling locations were chosen in order to compare different heavy metals concentrations caused by pollution. A group of nine animals in each place was collected along the mid-tide level in the intertidal zone during low tide. These animals were randomly distributed in three different samples, in order to avoid individual variation. Care was taken to ensure that the individuals were of quite a similar size, because some investigations have shown that the accumulation of heavy metals is dependent on specimen size.[11] All animals were washed with seawater. To assess the heavy metal contamination in Vigo estuary, seawater samples were collected 15–25 cm below the seawater level into 250 mL polyethylene bottles. Animal and water samples were transported to the laboratory at 4°C, and invertebrates were kept alive for 48 h in clean estuarine water to purge their digestive systems, in order to be able to measure only the heavy metals biologically deposited in their tissues.[12] Once purged, animals were killed by freezing.

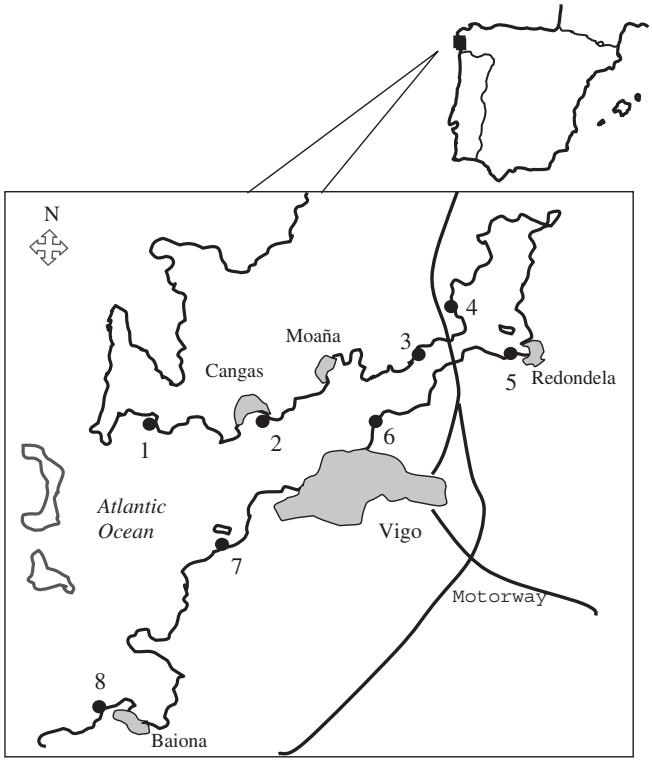

Figure 1. Localization of the sampling points along the Vigo estuary (Galicia, NW Spain).

Sample Preparation

Heavy metal analysis was carried out according to the method proposed by Melgar et al.[13] Limpets were extensively washed with ultrapure water, in order to remove adhering rests that could interfere with the determinations. Specimens from each sample site were divided into two portions: soft parts and shell. Each separate part was homogenized and dried at 105°C for 6 h. Approximately, 2 g aliquot of homogenized dry sample was placed in a porcelain crucible and ashed in an oven at 425–440°C for 15–40 h, till white ashes and constant weight. The final ashes were weighed and transferred into a 25 mL volumetric flask making up the level with 0.1 N HCl. The analyses were conducted in triplicate. The average weight and dry matter percentage for each sample were calculated.

Analytical Method

The concentration of four metals (Zn, Cd, Pb, and Cu) in seawater and limpet shell and soft tissues was measured by differential pulse anodic stripping voltammetry (DPASV), using a Metrohm Model VA 693 processor coupled to a Model VA 694 three-electrode stand. The auxiliary electrode was a platinum rod, the reference electrode Ag/AgCl/KCl (3 mol/L) and the working electrode an HMDE. The metal standard solutions (1000 mg/L) were obtained from Merck. Solutions of lower concentrations were prepared by dilution of the stock solution immediately before use. Acetate buffer, with CH_3COOH (2 mol/L) and NH_3 (1 mol/L), pH 4.6, was also used (Suprapur Grade Merck). Triply distilled mercury was used. A marine limpet sample volume of 5 mL diluted in the same volume of water and 0.5 mL of acetate buffer were introduced into the polarographic vessel. For water samples, the polarographic analysis was made using 10 mL of acidified water added with 0.5 mL of acetate buffer. The analytical conditions were: de-aeration time:180 s; accumulation potential: −1150 mV; accumulation time:120 s; voltammetric sweep: from −1150 to 50 mV; sweep rate: 20 mV/s; and pulse amplitude: 50 mV.

The determination limit was 1 μg/L (1 ppb) for Zn and Cu and 0.1 μg/L (0.1 ppb) for Cd and Pb. Precision and reproductibility of the method were determined by analyzing 10 replicates from one representative sample and also by calculating the coefficient of variation, which was 3.20%. According to these results, the method can be considered reproducible and precise. The epiphytic lichen *Evernia prunastri* (L) *Ach*. (IAEA-336) was used as the reference material. Quantification was carried out using the standard addition method (two additions per measurement). The final results are expressed in ppm (mg/kg) dry weight (dw) of sample. A linear regression statistical analysis was established in order to determine the correlation among the different heavy metals concentrations corresponding to the two portions isolated from the marine limpets.

RESULTS AND DISCUSSION

The results corresponding to the seawater analysis of the different samples are shown in Table 1. In general, the analyzed heavy metals showed increasing

Table 1. Heavy metals analysis (mean ± SD) of the different seawater samples.

Sampling locations	Metals (μg/L)			
	Zn	Cd	Pb	Cu
1	40.02 ± 7.28	0.01 ± 0.003	0.17 ± 0.05	0.14 ± 0.05
2	40.81 ± 10.13	0.01 ± 0.001	0.33 ± 0.05	0.26 ± 0.07
3	38.47 ± 5.29	0.01 ± 0.002	0.96 ± 0.09	0.52 ± 0.20
4	52.85 ± 6.04	0.03 ± 0.004	1.51 ± 0.19	0.72 ± 0.16
5	49.12 ± 5.01	0.02 ± 0.001	2.05 ± 0.20	0.88 ± 0.23
6	51.06 ± 8.21	0.01 ± 0.003	1.06 ± 0.22	0.12 ± 0.04
7	50.26 ± 6.30	0.01 ± 0.002	0.98 ± 0.16	0.35 ± 0.09
8	48.95 ± 4.89	0.05 ± 0.004	0.75 ± 0.19	0.26 ± 0.10

concentrations in seawater samples when the sampling point was situated in inner part of the estuary zone, with maximum values at sampling points 4 and 5. It must be noted that Cd results corresponding to sampling point 8, situated next to the open sea, reached the maximum concentration (0.05 ppm), directly related to a very important Zn content, about 49 ppm. In this case, as there is such a great correlation between the cadmium content in seawater and marine limpet, we can infer that it could have been caused by a punctual contamination. The existence of some contamination with this heavy metal connected with the sample manipulation was discarded, due to the existence of an important cadmium concentration in limpet samples, too, as shown below.

In general, the pattern may be the result of local anthropogenic sources, a mixture of contaminated sediments with relatively clean marine sediments and/or the release of metals into the water as a freshwater and seawater combination.[14]

Heavy metal (Pb, Cu, and Cd) concentrations in seawater samples were quite similar to values referred to in other regions of the world coastal waters.[15–17] On the contrary, Zn concentrations determined in seawaters from the Vigo estuary showed increased values when compared to other world areas. Except for water samples from Southern Bight (North Sea), where a maximum of 35.6 μg/L were quantified, other already studied Zn contents in seawater samples varied from 0.12 to 10.58 μg/L,[16] whereas the Zn values obtained from water samples from the Vigo estuary represented an average of 46.44 μg/L, some samples getting a figure as high as 50 μg/L. These results call us for a constant monitoring of this aquatic system to regulate the metal disposal in this area, as Vigo estuary is a world-wide known area for fishery business and edible seaweed culture.

The Zn contents in the limpet shells sampled ranged from 3.0 ppm at sampling point 1 to 7.6 ppm at point 8, as it is shown in Fig. 2(A). Even though sampling point 1 corresponds to an open sea area, where Zn-contamination was expected to be low, point 8 was situated in a similar area, and so, higher Zn-contamination seems to be directly connected with some special conditions of heavy metal accumulation in limpet shells. This fact could be directly related to the important amount quantified of this heavy metal when the seawater analysis was carried out, as shown in Table 1. On the contrary, these same Zn values referred to limpet soft tissues

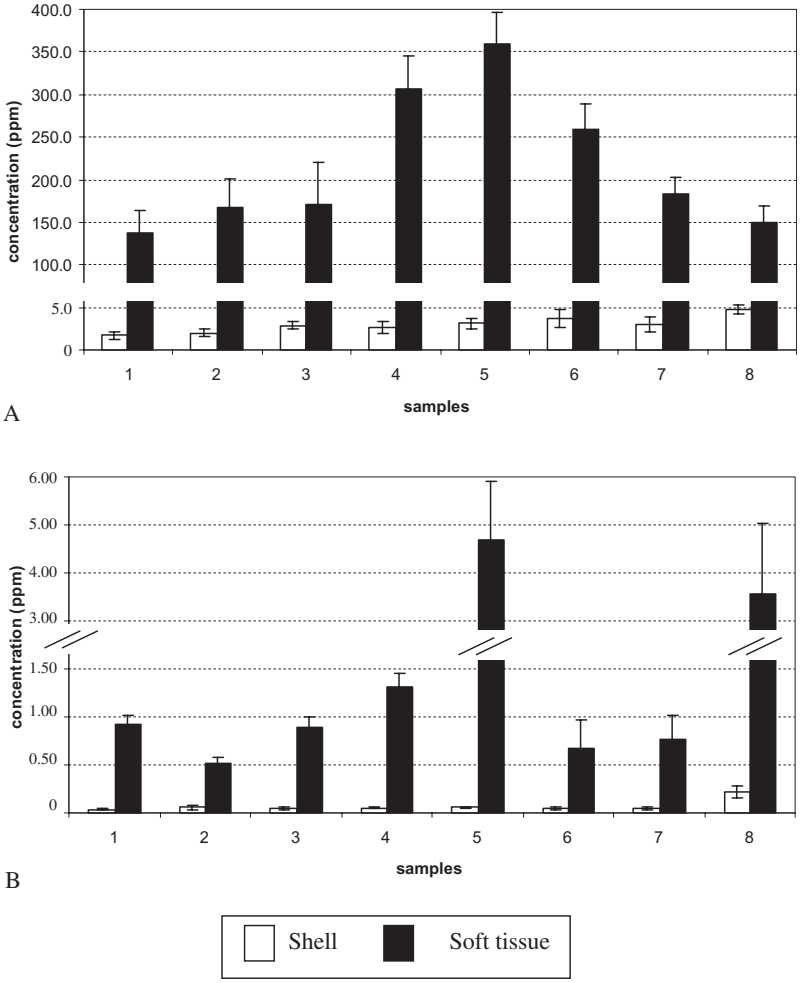

Figure 2. Zinc (A) and Cadmium (B) levels in soft tissues and shells from limpet, *Patella vulgata* L.

varied between 91.0 ppm at point 1 and 429.7 ppm at point 5, situated deep in the estuary system, and next to a dockyard effluent. In this case, a clear relationship between geographical localization and heavy metal content could be established, thus explaining the high Zn content determined in seawater analysis. It must be noted that Zn concentrations in limpet soft tissues reached up to 8 folds those in seawater samples analysis.

As it is represented in Fig. 2(B), limpet shells showed quite similar Cd concentrations in all the samples, with a minimum of it at sample point 1, where only 0.04 ppm was quantified. Curiously, higher Cd concentrations were determined at sample point 8, reaching up to 0.22 ppm, which represents more than 5 folds the lowest Cd concentration. This fact could be related to highest Zn concentrations in shells, which were also referred in this same sampling point. However, when Cd

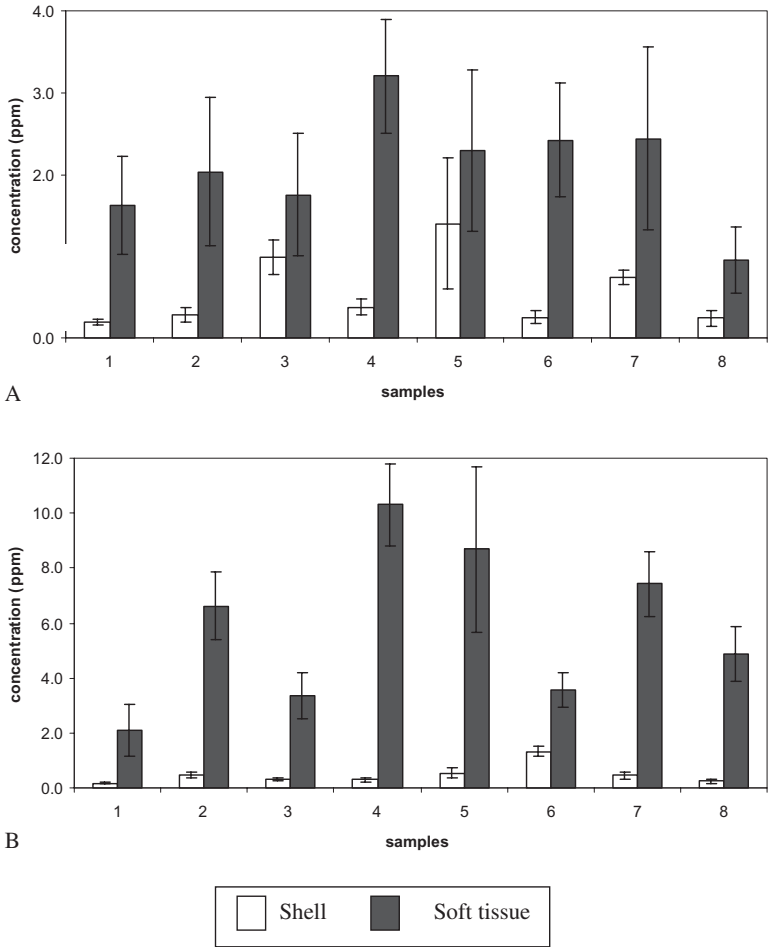

Figure 3. Lead (A) and Copper (B) levels in soft tissues and shells from limpet, *Patella vulgata* L.

content was determined in limpet soft tissues, the highest Cd concentration was observed in station 5, with 5.6 ppm. It should be noted that, for this metal, station 8 represented the second highest concentration (3.5 ppm). The concentration factor of Cd with regard to seawater samples in this sampling point was approximately 80 folds.

Lead concentrations in shell and soft tissues are shown in Fig. 3(A). Lead concentrations in shell showed an irregular distribution in relation to the sampling point, the maximum corresponding to some areas situated deep in the estuary (points 3 and 5), and lead levels decreasing towards the open sea. Sampling sites 4 and 6 showed abnormally low levels when compared with seawater analysis. As it was observed with Zn and Cd, the lowest Pb concentrations were found in station 1, with only 0.19 ppm, whereas station 5 showed concentrations over 1 ppm. It must be noted that Pb concentration in soft tissues was higher in point 4 (upper to 3 ppm),

whereas only 0.95 ppm were quantified in sampling point 8. However, in general terms the highest Pb concentrations corresponded to points from 4 to 7, whereas open sea areas corresponded to lower Pb contamination (an average of 1.3 ppm). The sources of this local Pb contamination could be connected with leaded petrol from cars and specially boats, and some local discharges from industries. In general, Pb constituted the least concentrated heavy metal in limpet soft tissues among the four studied elements (an average concentration of 2-folds).

In Fig. 3(B) the results corresponding to the Cu determination are presented. Once again, lower Cu levels in limpet shell were determined in samples from point 1 onwards, with only 0.1 ppm, whereas the sample from point 6 reached up to 1.3 ppm. On the other hand, soft tissue levels reached a maximum of 10.3 ppm at sampling point 4, five folds higher than those corresponding to the lowest levels, where only 2.1 ppm were quantified. In both Cu determinations, it can be seen how high the concentrations whose sampling zones are situated in inner part of the estuary can get, whereas sampling points located next to the open sea (points 1 and 8) presented the lowest concentrations. This special distribution was detected in Pb and Cd levels, but specially with Zn soft tissues, too. To this effect, it can be determined a direct relationship between the sampling localization and a clear increase in the heavy metal concentrations, at least as far as limpet soft tissues are concerned. This fact could also be directly connected with increasing levels of environmental contamination too, whereas heavy metal contents in shells are more related to long-time accumulation along life-cycle, and so, they do not seem to be reliable indicators of seawater quality. A direct correlation between Cu concentration in seawater samples and limpet soft tissues was observed (10-folds concentration), whereas no correlation could be established when studying Cu concentration in shell.

When linear regression statistical analysis of correlation was done (Table 2), high statistically significative correlation was found between variables corresponding to Zn and Cd contents in both soft tissues ($p < 0.01$) and shells ($p < 0.01$), and especially between Zn and Cu contents in limpet soft tissues ($p < 0.001$). No significant correlations were determined when the contents of each separated heavy metal in soft tissues and shell were compared.

Directly referred to mollusc heavy metal concentration, and as other investigators have already established,[18,19] higher concentrations of heavy metals were generally observed in the soft tissues of limpets compared with those determined in shells. This fact was patent in all the analyzed heavy metals in our samples, and especially when Cd and Zn determinations were done, with differences of 93.6 and 86 folds respectively. Curiously, in both cases, the highest differences were found at station 5. On the contrary, Gobert et al.,[19] working with the mussel *Mytilus edulis*, established that when Pb biomonitoring was carried out, the measuring of this heavy metal concentration in shell could be of particular interest, because shell concentration of Pb was greater than that from soft tissues, whereas for the rest of the analyzed heavy metals more important concentrations were quantified in soft tissues samples.

Paek et al.[16] working with *Littorina brevicula* determined that Cd, Cu, and Zn levels in soft tissues were directly correlated to the surrounding contamination levels. On the contrary, Langston and Zhou[20] working on *Littorina littorea*, determined its unsuitability as a specific indicator of Cu and Zn contamination, suggesting that

Table 2. Correlation matrix of the heavy metal content in limpet shell and soft tissues.

		Zn soft tissues	Cd soft tissues	Pb soft tissues	Cu soft tissues	Zn shells	Cd shells	Pb shells
Correlation	Zn soft tissues	1						
	Cd soft tissues	0.558	1					
	Pb soft tissues	−0.133	−0.161	1				
	Cu soft tissues	0.613	0.346	0.170	1			
	Zn shells	0.037	0.392	−0.007	0.034	1		
	Cd shells	−0.15	0.274	−0.132	−0.076	0.656	1	
	Pb shells	0.544	0.538	0.128	0.317	−0.030	−0.258	1
	Cu shells	0.232	−0.102	−0.011	−0.016	0.105	−0.255	−0.018
Significance (Unilateral)	Zn soft tissues							
	Cd soft tissues	0.002						
	Pb soft tissues	0.267	0.225					
	Cu soft tissues	0.001	0.049	0.213				
	Zn shells	0.433	0.029	0.488	0.438			
	Cd shells	0.242	0.098	0.269	0.363	0.001		
	Pb shells	0.003	0.003	0.275	0.066	0.445	0.112	
	Cu shells	0.137	0.318	0.480	0.471	0.312	0.115	0.467

it could be related to a certain capacity of these animals to regulate the cellular level of such essential metals. In this case, the difference between species to regulate heavy metal contents in their bodies could be determined by different levels of regulation or tolerance to these metals.

When studying the distribution of heavy metals in rock-shell, *Thais clavigera*, Han et al.[21] established by means of a linear regression analysis that a significant increase in Zn concentration was also associated with an increasing Cu concentration. Other authors have demonstrated, working with *Mytilus galloprovincialis* that it could be established a good correlation index between Pb levels in the soft tissues and the nacreous shell, but not to the rest of analyzed heavy metals (Co, Cr, Cu, Ni, and Zn).[19] Nevertheless, the interest of comparative studies between different mollusc species is relative. Heavy metal accumulation is different for each of these mollusc species, and lack of differences must be only ascribed to large individual variations more than to similarity in accumulation patterns.[15] This can be observed when comparing heavy metal contents from different edible mollusc species from the Pontedeume estuary.[22] So, when Pb was determined, 8.0 μg/g dry weight was the amount quantified in soft tissues from *Cerastoderma edule*, 74.0 in *Mytilus edulis*, and 81.8 in *Venerupis decusata*, always in the same sampling area.

It must also be taken into account that changes in heavy metal contents in different animal species like mussels, depending on the sampling time due to different biological activities in the year have been reported.[23] So, seasonal variations in metal content have been proved to be more marked in animals collected in coastal regions under an important flow of population or contamination when compared with stations situated in relatively unpolluted areas.[24] This fact could be one of the

most important parameters, to explain the concentration of pollutants in these animals. To this effect, it should be of interest to take that into account in future studies, in order to clearly establish changes in limpet heavy metal contents along the year. So, it can be concluded that for long-term studies on ecotoxicology a main interest would be to collect limpets throughout the year.

CONCLUSION

A direct relationship between heavy metal concentrations in seawater and limpet soft tissues was found in the Vigo estuary area, the levels of Cd and Zn being the ones that showed the greater spatial differences. In this sense, because of their tendency to concentrate pollutants of various kinds from the environment, certain marine molluscs have been found to be useful as indicator organisms, as the metal concentration in their organisms reflects ambient metal bioavailability. This fact is associated with the lack of mollusc movement, which may allow these organisms to integrate greater amounts of heavy metals. In the present work, this fact has been observed, indicating the possibility of using marine limpet in future biomonitoring programs on the Spanish coast.

Moreover, as some concern has been raised regarding the public health significance of the presence of heavy metals in shellfish, this kind of works can help to assist local and state health services in their deliberations concerning such contaminants in shellfish.

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