Trace metals in molluscs from the Beagle Channel (Argentina): a preliminary study

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Abstract

Individuals of *Mytilus chiloensis* (Hupè, 1854) and *Nacella magellanica metalica* (Rochebrune and Mabille, 1885) were evaluated as possible biomonitors of trace metal baseline contamination in the Beagle Channel, Tierra de Fuego (Argentina). The selected species have the necessary prerequisites for use as biomonitors. They are well distributed in all coastal areas of the Beagle Channel and other regional seas, they are easy to identify and available all year round.

The first aim of this preliminary survey is to evaluate the concentrations of Cr, Cu, Pb and Zn present in soft tissues of the selected species in order to have more information on the use of these selected species as possible cosmopolitan biomonitors. Samples were collected in seven stations situated along 170 km of the coast in the tidal zone. The validity of these two species as bioaccumulators was tested.

Significant differences between metal concentrations in molluscs from different stations were tested by ANCOVA on $\log_{10}$ transformed data with body weight as covariate. Multiple comparison tests (MCT) were conducted when significant differences were detected among the stations.

In *M. chiloensis* metal concentrations decreased in the order: Zn > Cu > Pb $\geq$ Cr while for *Nacella* metal concentrations decreased in the order: Zn > Cu > Cr > Pb showing good bioaccumulation ability. At present, they can be considered as good candidates as trace metals biomonitors for the studied area.

From all the obtained data and statistical analysis (ANCOVA, MCT) the results showed clearly that there is no one site univocally more contaminated (with clearly high levels of metals accumulation in biomonitors) than any other. Thus the possible hypothesis of the Ushuaia Harbour as being the most contaminated site must be reconsidered. Metal concentrations recorded may be used for background levels for intraspecific comparison within the Patagonian seas.

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1 Introduction

The use of biological species in the monitoring of trace metals in coastal areas allows evaluating the contaminants bioavailability or the effects of contaminants on marine organisms. Molluscs are among the organisms most used as bioindicators for trace metal pollution in biomonitoring programmes. Limpets and particularly bivalve molluscs can accumulate and integrate concentrations of several metals in seawater for relatively long intervals. They also assimilate trace metals from their food and from the ingestion of inorganic particulate material [1-4]. The levels of heavy metals accumulated by marine organisms are also a function of many factors such as temperature, salinity, diet, spawning, seasonal variations [5] and can also constitute a potential hazard for humans and other mammals. This is because some molluscs and limpets constitute a seafood group of particular interest.

The main advantage of the biomonitoring approach is a remarkable reduction in time and costs, consumed in frequent analyses of abiotic matrices of water and sediment samples. Bioaccumulators must have necessary requirements: they must be cosmopolitan and available all year round; they must accumulate the pollutant without being killed by the levels it comes in touch with; they must present a high concentration value of the contaminant and must be easy to sample and to preserve. Above all they must have a contaminant concentration that could be easily correlated with the concentration in the surrounding environment [5-7]. Mussels filter the surrounding water and they constantly accumulate metals in their tissues. Besides, they are easy to collect and to identify. *Mytilus chiloensis* (Hupè, 1854) is well distributed in South American seas (i.e. Beagle Channel, Strait of Magallanes, etc.); also a limpet *Nacella magellanica metalica* (Rochebrune & Mabille, 1885) is very abundant and it constitutes a good candidate as a possible biomonitor of trace metals in seawater. It lives on rocky substrata of tidelands at relatively shallow depths and tolerates fairly long periods of time outside of water. It eats algae and vegetable deposits that it scratches from the rocks. Moreover, it is important to underline that this herbivorous gastropod generally takes metals principally from the diet [8]. These molluscs are also relevant from the nutritional point of view. In fact they constitute a popular food in the studied areas.

The aim of the preliminary present study was to have more information on the use of these selected species as possible cosmopolitan biomonitors of Cr, Pb, Cu and Zn present in the Beagle Channel sea ecosystem.

2 Materials and methods

Beagle Channel is a strait in Tierra del Fuego, near the southern tip of South America. The channel has high ecological relevance and is about 240 km in length and between 5 and 14 km wide. It separates Isla Grande de Tierra del Fuego from several smaller islands to the south. The Beagle Channel was named for the British ship *Beagle,* in which Charles Darwin explored the area (1833–34).

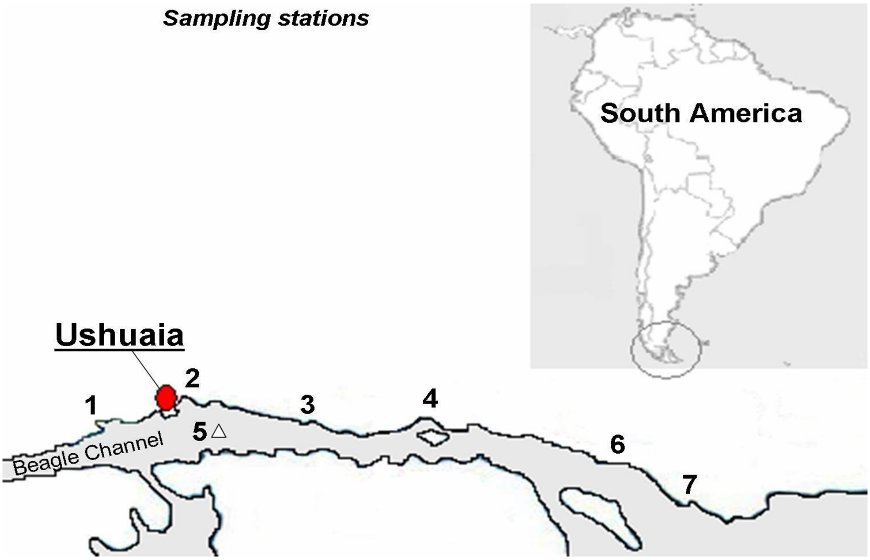

Figure 1: Sampling stations: 1) Lapataia Bay, 2) Ushuaia Harbour, 3) Punta Paranà, 4) Brown Bay, 5) Bridges Island, 6) Este Bay, 7) Punta Moat.

The molluscs have been sampled in February 2005 in 7 stations situated along of 170 km of the coast of the Beagle Channel (figure 1).

Individuals of *Mytilus chiloensis* (Hupè, 1854) (n=202) and *Nacella magellanica metalica* (Rochebrune & Mabille, 1885) (n = 105) have been picked in the tidal zone; then they were immersed (t = 24 hours) in filtered seawater to be purified.

Subsequently, the soft parts were taken out of the shell using tools (hammer and spatula) made exclusively of plastics, so as to prevent metal contamination, and then they were rinsed with deionized MilliQ water, in order to remove every residue of shell. Samples were placed in polyethylene bags, ice deep-frozen and transported to the laboratory.

Each sample of the mollusc previously homogenized (400-800 mg) was treated with 8 ml of 70 % (w/w) nitric acid Suprapur (Merck) and 2 ml of 30 % (w/w) hydrogen peroxide Suprapur (Merck) in PTFE vessels. The microwave digestion system MDS-2000 provided by CEM was used for the mineralization process.

Heavy metals in mollusc species were determined using a Shimadzu 6800 Atomic Absorption Spectrometer coupled to different atomic vapors generators depending on the analytical concentration. A graphite furnace accessory GFA-6000 and an autosampler ASC-6000 was employed for Pb and Cr measurements and an air/acetylene flame was used for Zn and Cu determinations.

The calculation of dry weight (d.w.) on the studied species (10 replicates for each location) was carried out through oven drying at 105 ºC until constant weight was achieved.

Traceability of results was obtained from the analysis of the certified reference material Antartic krill MURST-ISS-A2 (Italian Research Programme in Antartica). The mean recovery percentages (five replicates) were: Cr = 98.1±1.0 %; Cu = 101.1±1.3 %; Pb: 96.5±0.6 % and Zn: 102.1±2.9 %.

The detection limits (LODs) based on three times the standard deviation of the blank (n=11) were: Cr = 0.0002 mg.L^{-1}; Cu = 0.020 mg.L^{-1}; Pb = 0.001 mg.L^{-1} and Zn = 0.010 mg.L^{-1}.

Significant differences between metal concentrations in molluscs from different stations were tested by ANCOVA on $\log_{10}$ transformed data with body weight as covariate. Multiple comparison tests were conducted when significant differences were detected among the stations. SPSS 13.0 software was used for statistical analysis.

3 Results

Table 1 shows mean metal concentrations obtained by the analyses of the molluscs. Mean levels in biota are referred to dry weight to reduce variability of measurements determined by habitat, life conditions, pre-treatment and conservation of organisms after sampling [5].

In *M. chiloensis* metal concentrations decreased in the following order: Zn > Cu >Pb ≥ Cr. For *M. chiloensis* the values obtained were in the range of 57.91-111.54 μg g^{-1} dw for Zn; 3.90-7.41 μg g^{-1} dw for Cu; 0.21-0.76 μg g^{-1} dw for Pb and 0.26-0.98 μg g^{-1} dw for Cr. Very few data are available on *M. chiloensis*.

Mean Cu and Zn levels determined in the Beagle Channel (5.78 μg g^{-1} dw and 80.64 μg g^{-1} dw respectively, see table 1) for *M. chiloensis* were higher with respect to those obtained for Strait of Magallanes (Chile) [9]. The comparison with the only other available dated study [10] was not possible because wet/dry weight ratio for *M. chiloensis* is not reported. We think that reporting data referred to dry weight basis is of paramount importance because this procedure can avoid high variability in the obtained data. However, if we compare our bivalves data with other *Mytilus* species from Mediterranean seas [1], in areas with low-medium contamination levels, we observe significantly (p = 0.05) lower mean values for Cr, Cu, Pb and Zn in the Beagle Channel.

For *Nacella* the values obtained were in the range of 62,12-76.15 μg g^{-1} dw for Zn; 6.77-11.87 μg g^{-1} dw for Cu; 0.66-2.48 μg g^{-1} dw for Cr and 0.11-2.13 μg g^{-1} dw for Pb. As to *Nacella* metal concentrations decreased in the order: Zn > Cu > Cr > Pb. Mean Pb levels for *Nacella* were clearly lower than Cr while for *M. chiloensis* the mean accumulation levels of Cr and Pb were very similar.

No data are available in the literature for metal accumulation in *N. magellanica metalica.* However, if we compare our results with data relative to other *Nacella* species collected in Magallanes Strait [9] we observe that our mean values (p = 0.05) for Cu and Zn have higher levels. Comparing also mean accumulation data from this study with other patellid limpet from Mediterranean sea (*P. caerulea*) [1], mean levels of Cu and Zn obtained were significantly lower (p = 0.05) in *Nacella* samples from the B. Channel.

To assess the existence of significant differences in the metal concentrations in molluscs, use of ANCOVA on $\log_{10}$ transformed data with body weight as covariate was taken into account. This approach was chosen because the high variability on individual weights of the collected samples. For *Mytilus*, normality and homoscedasticity were checked, and resulted to be substantially improved by the log transformation with the exception of Pb values.

Table 1: Concentrations of metals (µg g^{-1} dry weight) in the soft tissues of *Mytilus chiloensis* and *Nacella magellanica metalica*, (mean ± s.d.).

Sampling station	**Individuals**	**Cr**	**Cu**	**Pb**	**Zn**
Mytilus chiloensis					
1	30	0.28±0.15	6.82±2.49	0.35±0.27	57.91±14.28
2	30	0.27±0.13	5.46±1.74	0.76±0.66	94.14±50.79
3	30	0.26±0.35	4.94±1.95	0.23±0.19	72.51±45.23
4	30	0.27±0.15	5.98±1.75	0.41±0.39	111.54±82.16
5	30	0.43±0.27	7.41±2.00	0.21±0.16	77.64±54.49
6	30	0.50±0.27	5.47±1.49	0.59±0.37	85.67±61.17
7	22	0.98±0.38	3.90±1.62	0.47±0.44	59.36±23.15
Total / Mean	**202**	**0.41±0.33**	**5.78±2.14**	**0.43±0.42**	**80.64±58.83**
Nacella magellanica metalica					
1	15	2.38±0.97	11.87±5.82	1.49±0.48	76.15±11,16
2	15	2.48±1.02	10.04±4.70	2.13±1.03	74.38±12.00
3	15	1.04±0.97	8.11±2.85	0.65±0.25	66.58±8.54
4	15	1.48±0.79	8.20±3.41	0.11±0.02	67.84±7.75
5	15	0.66±0.47	7.48±2.37	0.47±0.25	62.12±8.62
6	15	1.91±1.66	10.32±4.57	0.41±0.33	70.18±13.55
7	15	1.66±0.79	6.77±1.82	0.43±0.21	63.86±6.74
Total / Mean	**105**	**1.66±1.16**	**8.97±4.13**	**0.81±0.82**	**68.73±10.87**

Fresh/dry weight ratio: 3,54 (*Mytilus*) and 4,88 (*Nacella*).

Parallelism of the regression lines between all sites was tested throughout at a *p* level of 0.05. For Cr and Zn (*M. chiloensis*) the condition of parallelism of the regression lines was met (F=0.865, sig.=0.522 for Cr; and F=0.339, sig.=0.915 for Zn), then ANCOVA was performed. Results showed significant differences in Cr and Zn concentrations in *M. chiloensis* between the studied sites (Tables 2-3).

Table 2: ANCOVA test for Cr between sites (*M. chiloensis*).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9,224(a)	7	1,318	19,204	,000
Intercept	3,503	1	3,503	51,053	,000
Weight	,503	1	,503	7,330	,007
Site	4,665	6	,778	11,332	,000
Error	13,311	194	,069		
Total	75,548	202			
Corrected Total	22,535	201			

Table 3: ANCOVA test for Zn between sites (*M. chiloensis*).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1,337(a)	7	,191	4,211	,000
Intercept	95,387	1	95,387	2102,252	,000
Weight Total	,147	1	,147	3,240	,073
Site	1,261	6	,210	4,630	,000
Error	8,802	194	,045		
Total	694,189	202			
Corrected Total	10,140	201			

For Cu (*M. chiloensis*) the parallelism condition was not met (F=3.405, sig =0.003) and then ANCOVA was not performed. From the valuation of different regression models (logarithmic, exponential, polynomial, etc.) resulted that Cu depends slightly from weight. Then, analysis of variance (ANOVA) was performed. ANOVA results showed that significant differences are present between sites for Cu accumulation in *Mytilus*.

The tests of normality and homoscedasticity for Pb resulted to be not improved by the log transformation. For this reason, a non-parametric Kruskal-Wallis test was conducted. Results showed significant differences for mean Pb concentrations in *M. chiloensis* between the studied sites (Table 4).

Multiple comparison tests (MCT) were conducted for Cr, Cu and Zn (Tukey HSD; Bonferroni; Tamhane; Dunnett T3 and Scheffè) and non-parametric MCT were also conducted for Pb (Games-Howell, Dunnett's C, Tamhane's T2) in order to know which station is more contaminated than another.

Table 4: Kruskal-Wallis test for Pb between sites (*M. chiloensis*).

	Log Pb
Chi-Square	42,214
df	6
Asymp. Sig.	,000

Results for Cr (*M. chiloensis*) showed that site 7 (Punta Moat) has mean values significantly higher (0.98 μg g^{-1} dw; $p = 0.05$) than the all other sites. The sites 5 (Bridges islands) and 6 (Este Bay) have similar Cr mean values and significantly higher than sites 1 (Lapataia Bay), 2 (U. Harbour) and 3 (Punta Paraná) and 4 (Brown Bay) but significantly lower than the site 7.

MCT conducted for Cu in *M. chiloensis* showed that site 7 (P. Moat) has lower mean Cu levels than the others with the exception of site 3 (P. Paraná) that has similar levels of site 7.

MCT conducted for Zn in *M. chiloensis* showed that sites 2 (U. Harbour) and 4 (Brown Bay) have mean Zn values significantly higher than the all other sites. Sites 1 (Lapataia) and 7 (P. Moat) have mean Zn concentrations lower than sites 2 and 4.

MCT conducted for Pb showed that sites 2 (U. Harbour) and 6 (Este Bay) has significantly higher mean Pb concentrations than the others with the exception of site 7 that showed average levels of Pb.

For *Nacella* normality and homoscedasticity were checked and resulted to be clearly improved by the log transformation for all metals. The same ANCOVA model was employed. Parallelism of the regression lines between all sites was tested and the condition of parallelism of the regression lines was met for all metals. Then, it was possible to use ANCOVA for testing differences in mean metal concentrations between sites.

Results of ANCOVA showed that there are no significantly differences between mean Zn and Cu concentrations for *Nacella* between the selected sites. Besides, ANCOVA showed significantly differences between almost a couple of sites for Cr and Pb for *Nacella* species. Then, MCT were conducted for these metals.

MCT conducted for Cr in *Nacella* showed that sites 1 (Lapataia Bay) and 2 (U. Harbour) have the higher Cr levels; the sites 4 (B. Brown), 6 (E. Bay) and 7 (P. Moat) have average Cr levels; the sites 3 (P. Paraná) and 5 (Bridges islands) showed lower Cr levels.

MCT for Pb in *Nacella* showed that site 4 (Brown Bay) has mean Pb concentrations significantly lower than the other sites. The sites 3 (P. Paraná), 5 (Bridges Islands), 6 (Este Bay) and 7 (P. Moat) have similar Pb levels and significantly lower than sites 1 (Lapataia Bay) and 2 (U. Harbor). The sites 1 and 2 presented the higher Pb levels.

In conclusion, from the reported statistical analysis we can observe that there is no one site more univocally contaminated than another for the studied trace metals in the selected biomonitors.

We have also tested the bioaccumulation ability of *M. chiloensis* and *Nacella* by using the *t*-student test (Table 5).

This study confirms that *Nacella* has clearly high bioaccumulation levels of Cr, Cu, Pb and Zn than *Mytilus* (figures 2-5).

Table 5: *t*-student test for all metals. Equal variances not assumed.

Variable	*t*-student	df	Sig. (2-tailed)	Mean Difference
Cr	-10,818	112,701	,000	-1,2525163
Cu	-7,407	133,711	,000	-3,1885070
Pb	-4,488	133,744	,000	-,3811073
Zn	2,976	230,020	,003	11,9064298

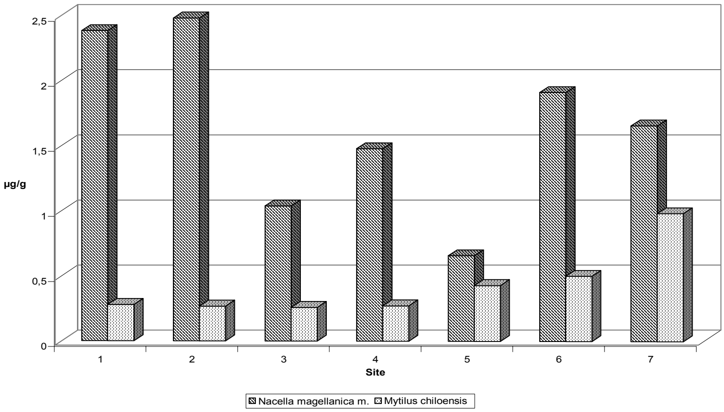

Figure 2: Cr concentrations in *Mytilus* and *Nacella* in the selected sites.

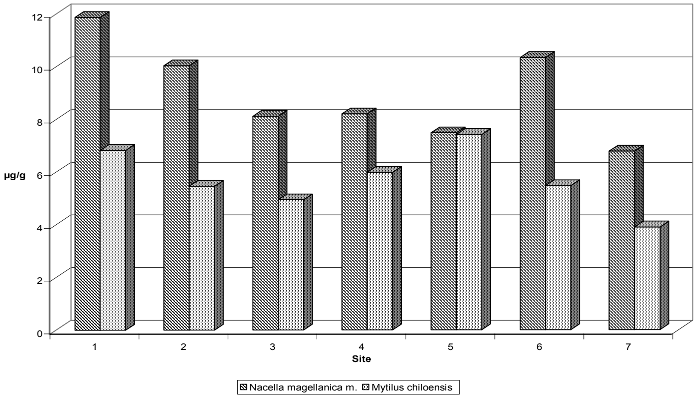

Figure 3: Cu concentrations in *Mytilus* and *Nacella* in the selected sites.

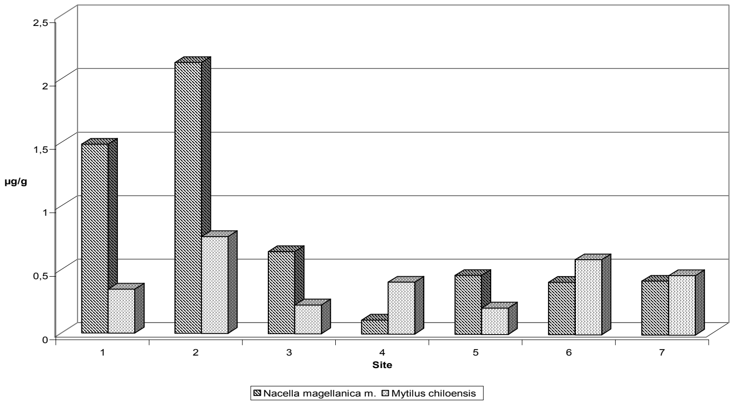

Figure 4: Pb concentrations in *Mytilus* and *Nacella* in the selected sites.

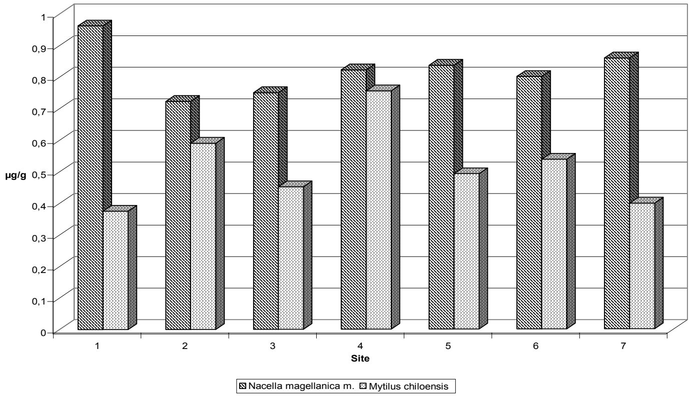

Figure 5: Zn concentrations in *Mytilus* and *Nacella* in the selected sites.

4 Conclusions

The use of the selected species turned out to be very valuable for the study of a coastal area with low levels of contamination. Then, *M. chiloensis* and *N. magellanica metalica* can be considered as potential biomonitors of trace metal pollution in the considered geographical areas. The selected biomonitors showed a very good ability to concentrate trace metals from seawater.

These two mollusc species have the necessary requisites: they are easy to identify and capture, they are sessile and sedentary, they are available all year round, and they are present along all coasts of the Beagle Channel.

Comparing our data with those available for *M. chiloensis* from the Magallanes Strait [9], the Beagle Channel bivalves showed higher Cu and Zn levels. However, this values are significantly lower ($p = 0.05$) than those of other *Mytilus* species from Mediterranean areas [1]. Comparing also our data with those available for other patellid limpets, our Cu and Zn accumulation levels for *Nacella magellanica metalica* were comparable to those of the Magellan Strait [9] and significantly lower than samples of Mediterranean areas [1].

Statistical analysis for *Mytilus* revealed that, as far as Cr is concerned, Punta Moat station presents higher Cr concentrations than the other sites. On the other hand the same site shows, with some exception, lower levels of Cu. Ushuaia Harbour and Brown Bay have Zn values significantly higher than all the other sites. Pb accumulation values for *Mytilus* showed that U. Harbour and Este Bay sites have, with some exception, higher Pb concentrations than the others.

By using *Nacella*, results showed that there are no significantly differences for Zn and Cu concentrations between the selected sites.

The Cr accumulation levels in *Nacella* were significantly higher in Lapataia Bay and Ushuaia Harbour. Punta Paraná and Bridges islands resulted with a lower Cr levels. The Pb accumulation levels for *Nacella* showed that Brown Bay has mean Pb concentrations significantly lower than the other sites. Lapataia and U. Harbour resulted with the highest Pb levels.

From the reported data and statistical analysis, it is clear that there is no one site univocally more contaminated (with clearly high levels of metals accumulation in biomonitors) than another. Thus, the possible hypothesis of the Harbour as being the most contaminated site must be reconsidered.

The follow-up of this study, extended to other biomonitors and contaminants along time and including metal determination in surrounding seawater, might further confirm these first results.

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