Metals and metalloids in mussels from the Argentine Patagonia

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Abstract: Mussels are bioaccumulative organisms that are used to monitor marine environments. The levels of 19 metals and metalloids were determined in *Mytilus edulis platensis* in three sites of the San Jorge Gulf: Bahía Solano (BS), Punta Maqueda (PM) and Km 3 (KM3). The limit values of contaminants As: 16; Cd: 3.7; Cu: 10; Ni: 3.4; Pb: 3.2 and Zn: 200, in $\mu g/g$ dry weight, were

not reached in the sampling sites, except for Cd in PM. The principal component analysis (PCA) showed a relationship between PM and high levels of Cd, between KM3 and high levels of Al, Cu, Pb and Zn, and between BS and high levels of Be, B, Sr and Ag. These results have contributed to the establishment of the state of situation in the spring of 2010 for 19 elements, which could be used in future studies of monitoring and assessment of the environmental risk owing to metal and metalloid contamination in the coasts of Argentine Patagonia.

Keywords: mytilus edulis; heavy metals; bioaccumulation; San Jorge Gulf.

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1 Introduction

National and regional biomonitoring represents a useful tool for quantifying and clarifying the current status of environmental health, and indirectly assessing coastal water quality and changes in the environment, which, in general, are due to anthropogenic causes. Monitoring programs use a great number of bioindicators 'sentinels' to detect temporal and spatial variations of chemical pollutants and to contribute to the knowledge of trends in marine contamination (Carro et al., 2004; Spada et al., 2013). Different organisms have been proposed as biomonitors, and bivalve molluscs, mussels in particular, are largely utilised as bioindicators because of their wide geographical distribution, easy sampling, sedentary nature, resistance to stress and sessile lifestyle (Cardellicchio et al., 2008; O'Connor, 2002; Conti et al., 2007).

Given that the aquatic is one of the most exposed to contaminants environments because they are the final receptacle of many anthropic discharges, the metals and metalloids studies are directed to protect the seaside ecosystem, in general, and the human health, in particular. Mussels are bioaccumulators and they are used to monitor marine environments. In these organisms, the phenomenon of bioaccumulation of elements from natural and anthropogenic contributions, and the biomagnification through the food chain, translates into a serious risk for large predators and, therefore, a potential risk for human health as the last link in the food chain (Gil et al., 1997; Roses, 1995).

On the Patagonic marine coast, there is a great amount of varied bivalve molluscs. In terms of ecology and feeding, the most important ones are *Aulacomyaatra*, commonly known as 'cholga' (ribbed mussel), and *Mytilus edulis platensis*, commonly called 'mejillón' (mussel). These communities cover large areas of the sea bottom and the rocky seaboard (Forcelli, 2000). Because they have a high commercial value and are important for the diet of certain social groups, their harvesting is a relevant activity on which the social and economic development of numerous towns on the region is based (Fajardo et al., 2016). It should be noted that mariculture has been declared an activity of provincial interest by the Honourable Legislature of the Province of Chubut in 1992 (Berón, 1999).

The purpose of this work was to determine metals and metalloids (Al, Ag, As, B, Ba, Be, Cd, Cu, Co, Cr, Fe, Mn, Mo, Ni, Pb, Se, Sr, V and Zn) in *Mytilus edulis platensis*, collected from three different sites of San Jorge Gulf, during the spring of 2010, and to compare whether there were significant differences in the concentrations of such elements among the different sampling sites: Bahía Solano, Punta Maqueda and the beach on Km 3.

2 Material and methods

2.1 Sampling sites

The San Jorge Gulf is extended over the coast of the provinces of Chubut and Santa Cruz, between Cape Dos Bahías and Cape Tres Puntas. It is a wide semi-circular gulf that has a sedimentary basin rich in hydrocarbons (Sciutto, 1995).

The bottom of the gulf currently consists of abrasion platforms (spits) including sea fossils, sand beaches, and in a small proportion, a kind of gravel known as Patagonian shingle formations (Isla, 2002).

Figure 1 Sample sites in the San Jorge Gulf: Bahía Solano, Km 3 and Punta Maqueda



The sampling sites (Figure 1) are placed at three spots of the San Jorge Gulf: the beach on the Km 3 (KM3), located at 3 km to the north of downtown Comodoro Rivadavia (a place that is close to the anthropic activity), Bahía Solano (BS) and Punta Maqueda (PM), located at 20 km to the north and 30 km to the south of Comodoro Rivadavia, respectively (both are places located far from human activity).

2.2 Sampling and sample preparation

During the spring of 2010, 40–60 mm sized mussel (*Mytilus edulis platensis*) specimens were collected manually to minimise the differences owing to age, size and filtration rate in the accumulation of the studied elements (Cubadda et al., 2001). They were extracted from the substrate, were kept in polyethylene bags and were taken to the lab at 5°C. They were washed with drinking water and, later, with ultra-pure water to remove sand and epibiota. The viscera mass was removed, dried at $50 \pm 5^{\circ}$ C for 24 h, and kept at –20°C until its processing.

2.3 Sample digestion

For the mineralisation, 100–200 mg of dry sample (100°C until constant weight) were exactly weighted, put in Teflon vessels (Parr Instrument Company, Illinois, USA), and 2 or 4 ml of HNO₃ (J.T. Baker ACS, EEUU) were added to them, respectively, depending on the sample weight. They were transferred to digestion bombs (Bomb Parr[®], Parr Instrument Company, Illinois, USA), and processed with microwaves for their wet mineralisation (1200 W for 1 min). Mineralisation products were put into polypropylene vessels at -20° C, until analysed.

The material used in the collection, pre-treatment and mineralisation of samples was washed with neutral detergent, rinsed with water, immersed in HNO₃ 50% v/v for 24 h and rinsed six times with ultra-pure water.

2.4 Analytical determinations

The elements were quantified in triplicate with an axial, simultaneous multi-element inductively coupled plasma spectrometer (ICP-OES), with solid-state detector and auto sampler Perkin Elmer Optima 5100 XL (Perkin Elmer, Ma. USA) provided with Winlab 32 Version 2.4 operating software.

2.5 Certified reference material

The trueness of measurements (expressed as bias) was tested by using the certified reference material (CRM) ERM-CE N° 278 (mussel tissue; Institute for Reference Materials and Measurements, IMMR, Brussels, Belgium). Six independent tests were performed on six different aliquots of the mentioned CRM. One sample of ERM-CE and blanks (reagents and digestion blanks) were included in each analytical batch. Results were in agreement with certified values and the standard deviations were low. Precision, expressed as intermediate precision, was not greater than 9%, using 10 independent replicates approach (Table 1).

The robustness was tested by introducing minimal changes in operating variables of the ICP-OES equipment (plasma gas flow, auxiliary gas flow, nebulisation gas flow, incident power, sample flow and variations in the reading of maximum wavelengths), and recovery was evaluated by analysis of 10 independent portions of the mentioned reference material. The uncertainty was evaluated from the analysis of its sources and quantification of each parameter of influence.

Element	Certificate (µg/g d.w.)	Found (µg/g d.w.)	Recovered (%)			
	$\overline{x} \pm S.D.$					
As	6.07	6.35 ± 0.386	105			
Cd	0.348	0.319 ± 0.009	92			
Cr	0.78	0.76 ± 0.371	98			
Cu	9.45	8.03 ± 0.434	91			
Mn	7.69	6.98 ± 1.46	91			
Pb	2.0	1.88 ± 0.01	94			
Se	1.84	1.80 ± 0.16	98			
Zn	83.1	73.5 ± 6.36	88			

Table 1Levels of elements ($\mu g/g$ dry weight) in reference material ERM-CE 278
(mussel tissue) n = 10

2.6 Statistical analysis

The descriptive results are expressed as an average and its standard deviation. To evaluate the association among the variables, the analysis of variance (ANOVA) method was used, with Tukey's Test as post-comparative test, considering the value of p<0.05 as statistically significant. Also, PCA was performed with standardised data. The statistical estimations were carried out with the InfoStat 2015 version (InfoStat, 2015).

3 Results and discussion

Table 2 shows the results of the metals and metalloids quantification in the analysed samples in microgram per gram of dry weight ($\mu g/g \, d.w.$), for the three sampling sites: Bahía Solano, Punta Maqueda and the beach on Km 3.

According to the ANOVA, the elements that did not show statistically significant differences in any of the three locations were: As, Mn and B. In KM3, the significantly high elements were Al, Pb and Zn, whereas V and Cr resulted significantly low. For all of them, there were no significant differences in the other two locations.

Cu and Ba resulted significantly high in KM3, intermediate in BS and significantly low in PM (p < 0.05). In the case of Cd, Co and Mo, values were significantly high in PM (Cd made the biggest difference), intermediate in BS, and significantly low in KM3 (p < 0.05), as well as in previous studies (Pérez et al., 2011, 2012).

The values of Fe and Ni resulted significantly high in PM, and there were no differences in the other two sampling sites. For Ag, the values were significantly high in BS, intermediate in KM3, and significantly low in PM (p<0.05). The values showed no differences in PM and KM3, but they were significantly low in BS. In the case of Be, the

values resulted significantly high in BS, intermediate in PM and significantly low in KM3 (p < 0.05), while in the case of Sr, the values were significantly high in BS, and there were no differences between the other two places (p > 0.05).

	BS	KM3	PM
		$\overline{x} \pm SD \; (\mu g/g \; d.w.)$	
Al	$357^b\pm46.6$	$826^{a} \pm 1.53$	$344^{b} \pm 52.4$
Ag	$1.60^a\pm0.00$	$1.00^{b} \pm 0.00$	$0.70^{\rm c} \pm 0,10$
As	$7.33^{a} \pm 0.81$	$5.90^{a} \pm 0.70$	$7.13^{a} \pm 0.83$
В	$21.5^{a} \pm 2.23$	$19.0^{a} \pm 0.20$	$19.4^{a} \pm 2.31$
Ba	$2.20^b\pm0.53$	$5.12^{a} \pm 0.17$	$0.67^{\rm c} \pm 0.12$
Be	$0.40^a\pm0.00$	$0.17^{c} \pm 0.02$	$0.20^{b} \pm 0.00$
Cd	$1.60^{b} \pm 0.20$	$0.17^{c} \pm 0.03$	$4.00^{a} \pm 0.53$
Cu	$4.33^{c} \pm 0.50$	$5.30^{a} \pm 0.30$	$4.20^{b} \pm 0.40$
Co	$0.50^b\pm0.10$	Nd	$0.87^{a} \pm 0.12$
Cr	$1.43^{a} \pm 0.18$	$0.62^{b} \pm 0.07$	$1.64^{a} \pm 0.27$
Fe	$355^b\pm 34.6$	$375^b \pm 13.2$	$458^a\pm24.6$
Mn	$11.3^{a} \pm 1.53$	$10.1^{a} \pm 0.30$	$11.1^{a} \pm 1.81$
Mo	$0.50^{c} \pm 0.10$	$0.44^b\pm0.00$	$0.80^{a} \pm 0.20$
Ni	Nd	Nd	$0.80^{a} \pm 0.20$
Pb	$0.22^b\pm0.05$	$0.87^a\pm0.23$	$0.22^b\pm0.05$
Se	$1.45^b\pm0.45$	$3.50^{a} \pm 0.10$	$3.80^{a} \pm 0.20$
Sr	$88.6^{a} \pm 4.51$	$78.6^{b} \pm 2.41$	$75.1^{b} \pm 3.89$
V	$4.30^{a} \pm 0.10$	$2.60^{b} \pm 0.00$	$4.20^{a}\pm0.00$
Zn	$100^{b} \pm 9.61$	$141^{a} \pm 6.60$	$113^{b} \pm 13.0$

Table 2Metals and metalloids concentration in Mytilus edulis platensis (µg/g d.w.)

 \overline{x} : Average; S.D.: standard deviation; d.w.: dry weight; Nd: Not detected. Different letters in the same row indicate a statistically significant difference (p < 0.05) among the sampling sites.

The PCA revealed that both principal components account for 100% of the data variability. In the biplot presented in Figure 2, it can be observed that with the first component (PC1) it is possible to differentiate the three locations, being the maximum values those recorded in KM3, the intermediate in BS, and the lowest in PM. On the *y*-axis (PC2), it is also possible to differentiate the three different locations, corresponding the maximum values to BS, the intermediate to KM3 and the lowest to PM.

PC1 accounted for the highest percentage of variability observed in the metal concentrations (62.9%). High values of PC1 are associated with high concentrations of Al, Cu, Pb and Ba, and at the same time with low values of V, Cr, As and Mn. It could be noted that Ag and Sr variables do not affect PC1, since they are nearly null. Mussels in KM3 showed higher PC1 values, which is to say that they are positively associated to increased concentrations of the previously mentioned elements (Al, Cu, Pb and Ba), and to Zn to a lesser extent.

PC2 accounted for a percentage of variability of 37.1%. High values of PC2 are associated to high concentrations of Sr and Ag, and low concentrations of Se and Fe.

In all, the observed relationships between the locations and the variables are: PM is positively associated to Cd and Mo; BS is positively associated to Be, B, Sr and Ag and KM3 is positively associated to Al, Cu, Ba, Pb and Zn.

Regarding the environmental quality of the sampled sites, Cantillo (1998) determined the maximum concentrations of the trace elements in mussels indicating contamination: As: 16; Cd: 3.7; Cu: 10; Ni: 3.4; Pb: 3.2 and Zn: 200, in μ g/g dry weight. Table 2 shows that the limit values for As, Cd, Cu, Ni, Pb and Zn were not reached in the three sampling sites, with the exception of Cd in PM, which exceeded the limit value of 3.70 μ g/g d.w.

Considering the legislation by the Swedish Environmental Protection Agency in the Baltic Sea (EPA, 2002), and according to Table 3, which establishes the reference values of the concentration of the elements that can be expected in different aquatic organisms to classify the quality of the marine ecosystems, BS and KM3 would be classified as Class 1 areas, areas little or no contaminated, and PM as Class 2 area, an area that is moderately contaminated owing to Cd.





 Table 3
 Concentrations set by the Swedish Environmental Protection Agency in the Baltic Sea

	Class 1	Class 2	Class 3	Class 4	Class 5
	Little or no contaminated	Moderately contaminated	Notably contaminated	Very contaminated	Extremely contaminated
-			$\mu g/g \ d.w.$		
Pb	<2	2–5	5-12	12–30	>30
Cr	<2	2–3	3–4	4–6	>8
Cd	<4	4–5	5-6.5	6.5-8	>8

It is worth noting that the observation of the increased values of Cd in PM agrees with previous studies of this investigation group, where increased levels of Cd were also found in PM, in red algae *Porphyra columbina* and *Mytilus edulis* (Pérez et al., 2005, 2010, 2011, 2012).

Most of the literature regarding the levels of heavy metals in the coasts of the Patagonia mentions sediments and suspended material (Gil et al., 1988, 1989, 1999; Giarratano et al., 2002). Available information on organisms is scarce (Gil et al., 1988; Pérez et al., 2005, 2007). A study carried out by Gil et al. (2006) in mussels from different sites of Southern Patagonia, such as Punta Loyola, San Julián, Puerto Deseado (Province of Santa Cruz), Rada Tilly, Comodoro Rivadavia and Caleta Córdova (Province of Chubut), shows some similarities. The concentrations of Cd (1.74–3.89 μ g/g d.w.) agreed with those found for this work. Nevertheless, the values of Pb (1.8–8.07 μ g/g d.w.) resulted considerably higher to those found in the three sampling sites of the San Jorge Gulf. In the case of Cu (4.37–10.5 μ g/g d.w.), the values are compared with the lowest data, and Zn concentrations (48.5–214 μ g/g d.w.) have medium values compared with this work.

In other Patagonic region, a study performed in the Strait of Magellan, Chile (Astorga España et al., 2007) reported levels of Cd, Cu, Fe, Zn and Mn in the same order of magnitude as this work, while the mean Se concentration was higher.

Conti et al. (2006) reported that Mytilus chilensis found in the Beagle Channel (Argentine) – similarly to what happens in the San Jorge Gulf – have concentrations of the following elements decreasing in this order $Zn > Cu > Pb \ge Cr$. In another study performed in the same area in 2005 and 2007, Conti et al. (2011) observed values in the range of 3.22-13.39 µg/g d.w. for Cu, 0.10-2.89 µg/g d.w. for Pb, 21.3-348 µg/g d.w. for Zn and 0.27–2.50 μ g/g d.w. for Ni. These values were observed to be higher than those found in San Jorge Gulf. Contrarily, the values of Cd were lower (0.13-2.30) when compared with PM, but higher than BS and although the latter zone receives wastewater effluents from human and fishing activity since is located near the port. Similar behaviour was described by Gutiérrez Galindo and Muñoz-Barbosa (2001) in Baja California, Mexico, where higher cadmium concentration ranges were reported in 'apparently clean' places (1.37-5.25 µg/g d.w.) compared with those places contaminated with wastewater discharges (0.60-2.13 µg/g d.w.). These authors attributed the difference to the oceanographic phenomenon of upwelling, whereas Conti et al. (2011) in the Beagle Channel explained this phenomenon by a higher bioavailability effect, which yields higher accumulation. Another possible explanation could be related to the presence of some concomitans that trigger Cd accumulations. However, this hypothesis needs further investigation.

Comparing the concentrations found at this study with those obtained (in other part of the world) by Mubiana (2005) in *Mytilus edulis* from Western Scheldt estuary, Netherlands, Cr and Cu values (5.30 and 17.0 μ g/g d.w., respectively) proved to be three times higher; Mn and Zn (42.0 and 565 μ g/g d.w., respectively) were four times higher; Cd (25.5 μ g/g d.w.) and Co (5.60 μ g/g d.w.) were six times higher; Pb (4.6 μ g/g d.w.) was five times higher and Ni (12.1 μ g/g d.w.) was 15 times higher than PM, the only place where Ni was detected. Finally, Fe concentrations were of the same order of magnitude (Mubiana et al., 2005).

Another work made in the Baie des Charleus, New Brunswick, Canada (Fraser et al., 2011), reported the same order of magnitude in the values of Cu, Zn and Fe in *Mytilus edulis*, with the results expressed in wet weight. However, the concentrations of Cd and Pb were, respectively, 6 and over 100 times higher than the ones found at this work.

4 Conclusions

From the global analysis of the results, it is inferred that the lowest levels of some elements, like Zn, Pb and Cu, are observed in PM. However, Cd levels were notably higher than in other places. On the other hand, KM3 was the area with the highest levels of Cu, Pb and Zn, probably associated to the port activity that is carried out nearby.

Nevertheless, the values set for mussels to define contamination in the sampling sites were not exceeded, according to the environmental parameters mentioned in the bibliography, with the exception of the high values of Cd in PM, which agrees with the Cd values found by this investigation group in another marine species (seaweeds) and other places in the Argentinian Patagonia. This aspect would merit additional research. Additionally, the PCA showed that it is possible to observe an association between PM and increased values of Cd and Mo; between BS and increased levels of Be, B, Sr and Ag; between KM3 and increased values of Al, Ba, Cu, Pb and Zn.

These results have *greatly* contributed to the establishment of the state of situation in 2010 for 19 elements, which could be used in future studies of monitoring and assessment of environmental risk owing to metal and metalloid contamination in the coasts of Patagonia.

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