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First records of metal concentrations in the Pacific oyster (*Crassostrea gigas*) from a Southwest Atlantic estuary

Noelia S. La Colla · Sandra E. Botté · Sandra M. Fiori · Eder P. Dos Santos · Ana C. Labudía

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Abstract The Pacific oyster (*Crassostrea gigas*) is one of the world's most widespread bivalves and a suitable species for biomonitoring metals in coastal environments. In the present research, wild individuals were collected from an Argentinian estuary and the coastal beaches nearby. The concentrations of eight metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were quantified in the soft tissues of the Pacific oyster. Among the metals, Cu, Fe and Zn reached the highest concentrations in the soft tissues over the rest of the elements. The results showed the highest values to be estuary related, with the beach site achieving the lowest values. These results possibly lie on the impact of human activities surrounding the estuary, as well as streams and rivers that outflow within it. Higher Cu and Zn levels, both port related, were mainly found toward the outer estuary. On the other hand, high levels of Cr, Fe and Mn were found toward the inner zone of the estuary, an area with sewage sludge from the cities located on the margins of the BBE. Regarding the potential risk to public health, Cu and Zn levels found in *C. gigas* were above national and international safety guidelines in 100% and 11% of the samples, respectively.

Keywords Pollution · Bivalves · Human health · Atlantic coast

N. S. La Colla (✉) · S. E. Botté · S. M. Fiori · E. P. Dos Santos · A. C. Labudía
Instituto Argentino de Oceanografía (IADO-CONICET-UNS), Florida 8000, B8000FWB Bahía Blanca, Argentina
e-mail: nlacolla@iado-conicet.gob.ar

S. E. Botté
e-mail: sbotte@iado-conicet.gob.ar

S. M. Fiori
e-mail: sfiori@iado-conicet.gob.ar

E. P. Dos Santos
e-mail: eder@iado-conicet.gob.ar

A. C. Labudía
e-mail: alabudia@iado-conicet.gob.ar

S. E. Botté · S. M. Fiori
Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670, B8000FWB Bahía Blanca, Argentina

Introduction

Pollution of marine environments has been progressively increasing over the years due to anthropogenic activities. The economic and demographic development of coastal regions (i.e., increase in urban, industrial, port settlements, as well as agricultural development) generates great loads of wastes, which are discharged into the waters. Among coastal environments, estuaries can be characterized by a slow rate of water exchange relative to their volume; therefore, they are more susceptible to pollutants inputs (Kennish 1997). These coastal ecosystems are complex, dynamic and have economical as well as ecological importance associated with their large marine biodiversity (Kennish 1997; Perillo 1995).

Within coastal marine systems, aquatic invertebrates tend to be highly vulnerable species as they might be exposed to several organic and inorganic pollutants (Kennish 1997, 2002). Metals are a serious threat because of their toxicity, long-time persistence, bioaccumulation and biomagnification in the food chain (Papagiannis et al. 2004). Through contaminated river flows, direct waste discharges, antifouling paints on ship hulls, atmospheric deposition, among others, these elements ultimately reach the coastal marine areas (Burioli et al. 2017). Benthic organisms, as inhabitants of estuaries, are known to take up and accumulate metals, both essential and nonessential, from water and sediment as well as from their food supply (Rainbow 1997, 2002; Wang and Fisher 1999).

Oysters are bivalve mollusks, epibenthic organisms of sessile nature with the ability to conduct filter feeding on suspended particles from surrounding waters, thus becoming a source of metal uptake in their bodies (Amiard-Triquet et al. 1992; Ettajani et al. 1992). The Pacific oysters (*Crassostrea gigas,* Thunberg, 1793) have the potential for rapid growth (Jonathan et al. 2017) and a relative resistance to pollutants (Goldberg 1986). *Crassostrea gigas* provide an excellent subcosmopolitan “sentinel” species as they are available all year round and can be easily collected. They are highly selective suspension feeders which feed on phytoplankton, suspended materials, sediments and aggregates consisting of high molecular weight substances, detritus, fecal pellets and microorganisms by filtering large volumes of seawater through their gills (Liu and Deng 2007; Ward and Shumway 2004). Moreover, due to their benthic and sedentary mode of life, oysters are exposed to environmental modifications (temperature, salinity, pollutants, etc.) with no possibility of escaping (Gagnaire et al. 2004). Bivalve shells increase attachment substrate; thus, at living at the same place, these organisms are ideal for monitoring changes in chemical concentrations at fixed locations (Rojas de Astudillo et al. 2002).

The Pacific oyster has been introduced for aquaculture worldwide, and invasive populations have frequently become established and spread outside cultivation farms, where they cause significant changes to coastal ecosystems (Chew 1990; Ruesink et al. 2005). *Crassostrea gigas* was introduced during the last quarter century to several South American countries, including Chile, Peru and Ecuador along the Pacific coast and Argentina and Brazil on the Atlantic (Melo et al. 2009; Orensanz et al. 2002). In Argentina, the species was introduced into Anegada Bay (40°S) in 1982, where aquaculture was soon abandoned; 10 years later, its first spontaneous population was recorded in the country (Borges 2006; Escapa et al. 2004; Orensanz et al. 2002). Since then, it has spread both northward (Bahía Blanca estuary 38°S62°W; Dos Santos and Fiori 2010) and southward (El Condor 41°S62°W; Roche et al. 2010). Invasive populations of *C. gigas* are associated with a potential risk to public health due to human consumption of oysters harvested from areas contaminated by domestic and industrial sewage wastes. Metal risks to public health include serious damage to kidneys, central nervous and immune systems (Antón and Lizaso 2002; ATSDR 2005).

Oysters from the Bahía Blanca coastal ecosystem are exposed to metals from the plankton (Fernández-Severini et al. 2013), the fraction of the particle-bound and dissolved metals available to filter-feeding species (La Colla et al. 2015) and particulate matter stored in contaminated sediments (Botté et al. 2010; Serra et al. 2017). Then, the current study focused on reporting, for the first time, the concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the soft tissues of *C. gigas* from the Bahía Blanca estuary and the nearby coasts of Argentina. The main goals of this research were: (1) to study the metal accumulation in the invasive populations of these sessile and filter-feeding organisms, considering their recent introduction into the Bahia Blanca estuarine environment (Dos Santos and Fiori 2010) and their exposure to environments with different degrees of anthropogenic impact; (2) to test differences between the sampling areas surveyed as a consequence of the differences in their surrounding environment, expecting to achieve higher metal values in tissues of oysters located in the both inner and outer estuary compared with the beach area, due to the anthropogenic impact of the industries and big cities located along the estuary; (3) to compare the levels of metals found in the soft tissues with the certified human consumption safety guidelines recommended by both international and national legislations.

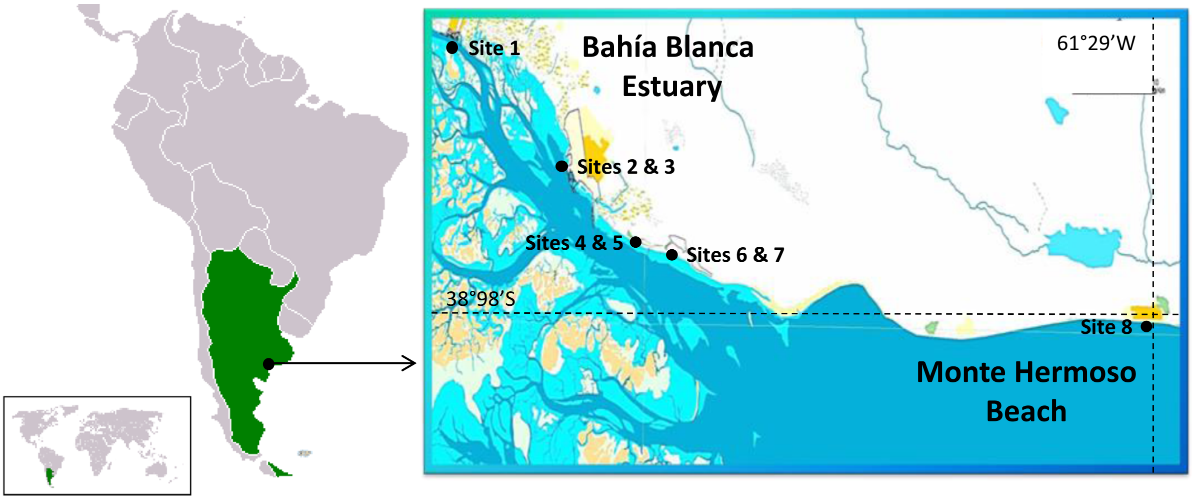

Fig. 1 Map of the southeast coast of the Buenos Aires province, showing the study area. Site 1 is located in Cuatreros Port area; sites 2 and 3 are located in Ingeniero White Port area; sites 4 and 5 are located in Belgrano Port area; sites 6 and 7 are located in Coronel Rosales Port area; site 8 is located in Monte Hermoso Beach area

Materials and methods

Study area

The Bahía Blanca estuary (BBE) is located in the southeast coast of the Buenos Aires Province, Argentina (38°45′ to 39°40′S and 61°45′ to 62°30′W) (Fig. 1). It is a mesotidal coastal plain estuary that extends over approximately 2300 km^2 and is formed by several tidal channels, extensive tidal flats with patches of low salt marshes, and islands (Piccolo et al. 2008).

Urban development, industries and agriculture areas are located in the northern shore of the Principal Channel, the main navigation channel from the BBE. The city of Bahía Blanca, with 300,000 inhabitants (INDEC 2010), is the most important urban center in the area and assembles a set of deepwater ports, including oil transfer buoys, trading docks and the largest navy base in the country. Several dredging programs in the main navigation channel enabled the settlement of one of the most important petrochemical poles in South America (Zilio et al. 2013). By 2002, the industrial area surrounding the petrochemical center embraced only nine industries, while in 2012 it included more than 135 industries (Sznaiberg 2012). Raw sewage discharges, insufficiently treated industrial effluents and runoff from nearby agricultural areas are the major sources of pollution to the estuary, raising concern about the ecological integrity of the estuarine system (Ferrer et al. 2000; Marcovecchio et al. 2008; Perillo et al. 2001; Tombesi et al. 2000).

Monte Hermoso (MH) is a coastal town located 120 km away from Bahía Blanca city (Fig. 1). The coast of MH is influenced by the plume of the BBE, but with an overall low degree of anthropic alteration (Berasategui et al. 2017). MH beaches are characterized by open sandy beaches of gentle slope supported by extensive sand dunes with minimum urban development (Delgado et al. 2012). The area is characterized by a mesotidal regime with semidiurnal tidal cycles, with mean tidal amplitude of 3.10 m (Menéndez et al. 2016).

Previous studies have indicated the presence of a variety of metals (mainly Fe, Mn, Cd and Cu) in mudflats and suspended sediments of the BBE (Botté et al. 2010; Marcovecchio et al. 2010; Serra et al. 2017), as well as accumulation in living organisms (La Colla et al. 2017, 2018; Simonetti et al. 2013). Reported values are similar to those found in other polluted estuarine environments, and metal concentrations achieved in the seawater fractions showed an increasing trend for some metals when compared to previous studies from the same estuary (La Colla et al. 2015 and references therein).

Sampling areas and sampling sites

Four harbor areas were selected from the BBE: Cuatreros Port (CP), Ingeniero White Port (IWP), Belgrano Port (BP) and Coronel Rosales Port (CRP). A fifth sampling area, located outside the estuary in the coastal beaches nearby, was selected: Monte Hermoso Beach (MHB). Oysters were sampled from eight sampling sites located in these five sampling areas (site 1 from CP; sites 2 and 3 from IWP; sites 4 and 5 from BP; sites 6 and 7 from CRP; site 8 from MHB) (Fig. 1).

CP is located in the inner part of the estuary, close to a small town. This area is influenced by the Sauce Chico river and the Saladillo de Garcia stream, which drain large extensions of agricultural fields (Limbozzi and Leitào 2008; Perillo et al. 2001). IWP is located in the middle estuary and close to the deepwater port area and the industrial area represented by oil petrochemical and chemical industries. Periodical dredging, artisanal and commercial fisheries, and oil and cereal cargo vessel traffic usually affect this area, as well as the sewage discharges of the Bahía Blanca and Ingeniero White cities. BP and CRP are located on the northern coast of the outer reach of the main navigation channel. BP is the most important naval base of Argentina (Cuadrado et al. 2001). CRP is impacted by urban effluents with scarce treatment from Punta Alta city and receives the influence of the harbor located adjacent to this city.

MHB is an area located in the continuous coastal fringe of the exposed sandy beaches of MH city. Its economy is mainly based on the exploitation of marine and coastal resources, firstly touristic activities and secondly artisanal fishery and is primarily impacted during midsummer period, due to the significant differences between permanent and occasional (summery) population. During the peak season, population increases from 6000 up to 60,000 persons (Rojas et al. 2014). This area is also influenced by agricultural–livestock activities.

Cleaning procedures

All material used during sampling and in the laboratory was previously cleaned according to internationally recommended protocols (APHA 1998). The conditioning procedure includes washing the material with nonionic detergent, rinsing them three times with tap water and then three times with deionized water. After that, the material is soaked for 24 h in a diluted acid nitric solution (5.0% HNO_3, MERCK) and finally rinsed three times with deionized water.

Sample collection and laboratory analyses

A total of 40 oyster samples were collected between 2013 and 2015, five samples collected at each sampling site. Oysters were manually harvested during low tide and carried to the laboratory facilities.

Of the collected oyster samples, the length of the shells was measured using a caliper. The dissection was performed in the laboratory with a stainless steel knife, and the complete soft tissue was removed from the shell and washed with distilled water to remove impurities. The wet weight of individual whole soft tissues was measured. Length and weight of each sample were measured to the nearest 0.1 mm and 0.1 g, respectively (Table 1). Tissue samples of oysters were not pooled, so each sample consisted of a unique individual oyster from which the whole soft tissues were homogenized, put in a polyethylene bag and frozen at − 20 °C until analysis.

Analytical procedure

Concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined based on the methods described by Botté et al. (2010) and with further modifications from La Colla et al. (2017). Whole soft tissues portions (0.50 ± 0.05 g, wet weight) were subjected to an acid predigestion with 5 ml of HNO_3 (65%, MERCK) for at least 3 h. Then, 1 ml of $HClO_4$ (MERCK) was added and the samples were put in a glycerin bath at 110 ± 10 °C for 72 h or until the volume was reduced to less than 1 ml. The acidic extracts were transferred to centrifuge tubes, and 0.7% HNO_3 was added up to 10 ml of a final dissolution. Metals were analyzed with a Perkin-Elmer Optima 2100 DV inductively coupled plasma-optical emission spectrometry (ICP-OES).

Quality assurance and quality control

The analytical method detection limit (MDL) for each metal (μg/g) was: 0.11 for Cd, 0.03 for Cr, 1.4 for Cu, 0.12 for Fe, 0.015 for Mn, 0.04 for Ni, 1.2 for Pb and 0.28 for Zn. Blanks of reagents were used simultaneously in each batch of analysis to corroborate the

Table 1 Characteristics of oysters (*Crassostrea gigas*) from the Bahia Blanca estuary and the coastal waters nearby

Sampling site	Mean total length (mm) (± SD)	Mean total soft tissues weight (g, w.w.) (± SD)
Site 1	65 (± 19)	13 (± 13)
Site 2	72 (± 4.8)	10 (± 2.2)
Site 3	61 (± 7.0)	6.3 (± 2.8)
Site 4	78 (± 16)	17 (± 4.6)
Site 5	110 (± 22)	44 (± 19)
Site 6	84 (± 28)	24 (± 19)
Site 7	80 (± 17)	13 (± 5.5)
Site 8	73 (± 12)	13 (± 5.6)

SD standard deviation, *w.w.* wet weight

analytical quality. All analyses were performed in duplicate, and the uncertainty based on one relative standard deviation of replicates was $< 15\%$. The analytical quality was tested against reference materials (mussel tissue flour R.M. N°6) provided by the National Institute for Environmental Studies (NIES) from Tsukuba (Japan). The concentrations found were within 85–115% of the certified values for all the measured elements.

Statistical analyses

All statistical analyses were carried out using INFOSTAT (free version http://www.infostat.com.ar). Metal concentrations reported as below the MDL were substituted by one-half the MDL for statistical analyses (Jones and Clarke 2005). Because of the small sampling size, no statistical analyses were performed when 50% or more of the metal concentrations under evaluation were below the MDL (EPA 2000). Normality and homogeneity of variance were tested with Shapiro–Wilk and Levene tests, respectively. One-way analysis of variance (ANOVA) was performed in “Results” section. When the assumptions of normality and homogeneity could not be met and there were no possible transformations, the Kruskal–Wallis ANOVA was applied (nonparametric *H* test). Significant results were analyzed a posteriori with the Tukey’s HSD test after an ANOVA or with the Conover scores after a Kruskal–Wallis test (Conover 1999). All these tests were performed to assess differences in metal concentrations between sampling sites and to test differences in oyster sizes between sampling sites. The oyster sizes were also used for statistical comparisons as a covariate in order to consider its influence on the analyses. Correlations analyses were implemented between the metal in the soft tissues of the oysters, as metals might be originated from similar sources and/or have similar reactivity toward environmental parameters.

Non-metric multidimensional scaling analysis (nMDS) was used to represent the sampling areas under study. The technique was based on triangular matrix using the Euclidean distance on standardized sample data from all metals analyzed. Differences between areas were tested by means of the one-way ANOSIM permutation test (Clarke and Warwick 1994) at a significance level of $p < 0.05$ and R statistic > 0.5. Similarity percentage analysis (SIMPER) was used to determine the metals that most contributed to the differences observed. These analyses were performed with PRIMER 6 (Clarke and Gorley 2006). Comparisons were made between sampling sites comprised within the inner zone of the BBE, the outer zone of the BBE and the sandy beaches nearby.

Principal component analysis (PCA) correlation based was undertaken in order to identify whether groups of sampling sites exhibited relationships across the metals. The correlative relationship between metals was simply compared using Spearman’s rank correlation of un-transformed concentrations.

Results

Metal concentrations

The metal concentration ranges in the soft tissues of *C. gigas*, considering all sampling sites, revealed the

Table 2 Mean concentrations and standard deviation of the metals Cd, Cu, Cr, Fe, Mn, Ni and Zn (in µg/g, wet weight) in the soft tissues of *Crassostrea gigas*

Sampling area	Sites	Soft tissues of *Crassostrea gigas* (mean value in µg/g, ± SD)													
		Cd		Cu		Cr		Fe		Mn		Ni		Zn	
CP	1	0.42 ± 0.10	a	38 ± 13	a, b, c	0.12 ± 0.048	a, b	34 ± 13	b, c, d	4.7 ± 5.2	a	0.087 ± 0.027	a, b	100 ± 47	b, c
IWP	2	0.50 ± 0.091	a, b	50 ± 12	c d	0.13 ± 0.086	a, b	83 ± 21	d	3.6 ± 0.79	a	0.16 ± 0.044	c	110 ± 26	b, c
	3	0.41 ± 0.085	a	24 ± 5.7	a	0.20 ± 0.12	b	110 ± 71	d	6.4 ± 4.1	a	0.14 ± 0.029	b, c	110 ± 19	b, c
BP	4	0.76 ± 0.052	d	49 ± 8.2	b, c, d	0.10 ± 0.038	a, b	46 ± 8.0	b, c, d	2.5 ± 0.51	a	0.12 ± 0.045	a, b	130 ± 25	c
	5	0.55 ± 0.12	a, b, c	55 ± 13	c d	0.078 ± 0.020	a, b	34 ± 8.0	a, b, c	5.7 ± 1.8	a	0.040 ± 0.015	a	170 ± 84	c
CRP	6	0.39 ± 0.052	c d	25 ± 5.2	d	0.075 ± 0.041	a, b	23 ± 8.7	c d	4.4 ± 1.9	a	0.073 ± 0.020	c	73 ± 23	b, c
	7	0.74 ± 0.10	a	60 ± 3.5	a	0.18 ± 0.057	a	55 ± 12	a	2.9 ± 0.59	a	0.18 ± 0.046	a, b	110 ± 3.1	a, b
MHB	8	0.70 ± 0.064	b, c, d	29 ± 2.8	a, b	0.096 ± 0.025	a, b	37 ± 22	a, b	3.4 ± 1.4	a	0.049 ± 0.016	a	42 ± 2.8	a

The concentrations are expressed according to the sampling sites and areas. Pb is not plotted since all values were below the limit of detection of the method

SD standard deviation, *CP* Cuatreros Port, *IWP* Ingeniero White Port, *BP* Belgrano Port, *CRP* Coronel Rosales Port, *MHB* Monte Hermoso Beach

^{a,b,c,d}Post hoc tests: letters indicate significant differences between sampling sites

following decreasing range order (in µg/g, wet weight): Zn 39–319, Fe 12–231, Cu 19–81, Mn 1.5–15, Cd 0.29–0.89, Cr < MDL-0.42, Ni < MDL-0.24 (Table 2). All Pb values were below the MDL. For most trace elements, values varied significantly depending on the sampling site ($p < 0.05$). One-way ANOVAs and post hoc comparisons (Tukey's tests) found the highest Cd concentrations in the sampling site 4 in comparison with the majority of the other sites ($F(7,32) = 12.4$; $p < 0.0001$), and with no statistical differences against sites 6 and 8 ($p > 0.05$). In the case of Cu, site 6 stood up as the sampling site with the highest mean value compared with the majority of other sites (i.e., 1, 3, 7 and 8) ($F(7,32) = 9.9$; $p < 0.0001$). Differences in the values for Cr were only found in site 3, with this site achieving higher levels compared with site 7 ($F(7,32) = 2.95$; $p = 0.0167$). As for Ni, sites 2 and 6 achieved higher values than the rest of the other sampling sites ($F(7,32) = 13.56$; $p < 0.0001$) with the exception of site 3.

Kruskal–Wallis ANOVA and post hoc tests were used for the analyses of the rest of the evaluated metals. Sites 2 and 3 achieved higher Fe values than sites 5, 7 and 8 ($H = 25.31$; $p = 0.0007$). Moreover, site 8 achieved the lowest Zn values ($H = 20.93$; $p = 0.0039$), with the exception of the sampling site 7, that did not proved to be any different ($p > 0.05$). Among Mn concentrations, no differences were found ($H = 13.32$; $p = 0.0645$) between sampling sites. Nevertheless, it must be taken into account that the p value was very close to 0.05. Finally, in the case of Pb, all the values were below the MDL and thus no statistical analysis could be performed.

Differences in metal concentrations became more evident within the estuarine area, at sampling sites located in IWP (i.e., 2 and 3), BP (i.e., 4) and CRP (i.e., 6). These four sampling sites were highlighted as the ones with the highest mean values for the majority of the metals. Moreover, throughout an nMDS (Fig. 2), the metal accumulation pattern was tested in relation to the characteristics of the sampling areas. Sampling areas were divided into three differentiated zones. The first zone, renamed "inner zone of the estuary," comprises the sampling areas CP and IWP (i.e., sites 1, 2 and 3). The sampling areas BP and CRP (i.e., sites 4, 5, 6 and 7) were renamed "outer zone of the estuary," and the sampling area MHB (i.e., site 8) was renamed "beach zone." Values of each group were averaged

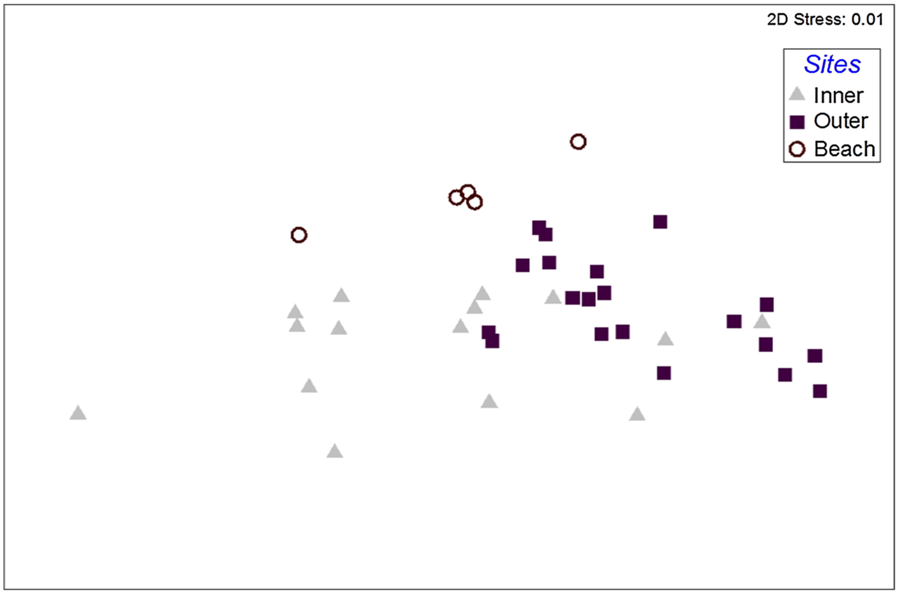

Fig. 2 Multidimensional scaling (nMDS) plots of metal accumulation between sampling zones. Inner sites (gray triangle): sites 1, 2 and 3; outer sites (filled square): sites 4, 5, 6 and 7; beach site (open circle): site 8

and differences were advised between the inner sites of the BBE against the outer sites of the BBE (ANOSIM R global 0.34; $p < 0.01$). The SIMPER routine showed that Fe and Zn were the two metals that mostly contributed to the observed differences (58% and 32% contributions, respectively). Differences were also found between the outer sites of the BBE and the beach. Nevertheless, no differences were found between the inner sites of the BBE and the beach.

As regards the PCA analyses, they identified two main factors explaining 72% of the total data variance. Pb was removed from the data matrix because its concentrations were all below the MDL. The first component (CP 1) accounted for 40% of the total variance, and the results showed that oysters sampled from sites 2 and 6 were clearly different from the others by its location on the positive side of CP 1 (Fig. 3). The high levels of Ni defined this separation. On the contrary, sampling sites 7 and 8 were encompassed by the high levels of Mn. The second component (CP 2) explained 32% of the total variance. The oysters from site 3 were located on the positive side of CP 2, which was characterized by high Fe, Cr and Mn concentrations. On the contrary, the oysters collected in sites 4, 5 and 6 were on the negative side of CP 2 and were characterized by high levels of Cd and Cu (Fig. 3).

Correlation analyses between metal values in oyster tissues were performed, and statistically significant positive correlations were found between Cu and Cd (r^2: 0.63; $p < 0.05$) and between Cu and Zn (r^2: 0.77; $p < 0.05$). Moreover, Ni was positively correlated with both Cr and Zn (r^2: 0.64; $p < 0.05$).

As regards health hazards, Cu and Zn concentrations in soft tissues of *C. gigas* exceeded the maximum permitted metal levels for bivalve mollusks according to national and international guidelines (Table 3). On the other hand, Pb concentrations in oyster tissues were always below the limit of detection, whereas Cr values never exceeded maximum concentrations during the study. There are no guidelines regarding maximum Fe and Mn permitted concentrations for bivalve mollusks.

Biological information

The size of oysters is one of the most important endogenous factors that could affect metal accumulation. The analyses of the samples revealed the following decreasing order in oyster sizes between the sampling areas: BP > CRP > IWP = MHB = CP. The Kruskal–Wallis ANOVA was implemented

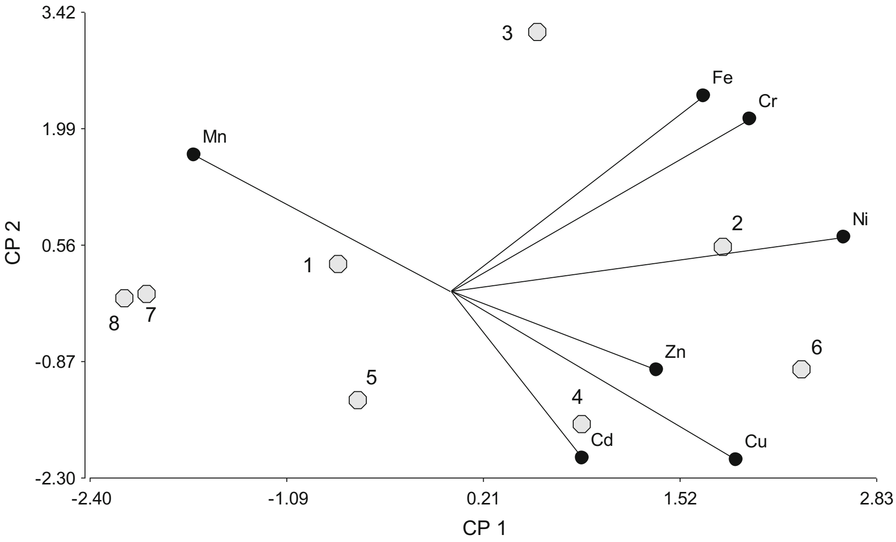

Fig. 3 Principal component analysis (PCA) conducted to identify whether groups of sampling sites exhibited relationships across the metals. Consider 40% of explication for axis 1 (CP 1) and 32% of explication for axis 2 (CP 2). The numbers correspond to each sampling site. Pb is not plotted since all values were below the limit of detection of the method

to test differences in the oyster sizes between sites. According to the results, site 5 from BP showed higher sizes compared with sites 1, 2, 3, 7 and 8 ($H = 15.16$; $p = 0.0339$) and similar sizes compared with sites 4 from BP and 6 from CRP. Nevertheless, the size did not showed covariance with each of the metal concentrations between sites ($p > 0.5$). Despite the efforts to reduce the high variability in oyster sizes, homogeneity in the oyster lengths was not reached. Nevertheless, the lack of influence of the sizes in the metal distribution might be related to the fact that there are other variables responsible for the metal variations between sites.

Discussion

This research reports the metal concentrations found, for the first time, in the soft tissues of the oyster *C. gigas* from the BBE and the coastal area nearby and relates the current results to the different sampling areas, within a coastal system exposed to different pollution processes (Arias et al. 2010; La Colla et al. 2015; Oliva et al. 2015). *Crassostrea gigas* is a filter-feeding organism in the position to respond to metals dissolved in water and associated with suspended particles and plankton (Amiard-Triquet et al. 1992; Ettajani et al. 1992). Moreover, the invasive populations of *C. gigas* are associated with a potential risk to public health, since they are widely consumed by local populations, without any further health or safety controls.

Fe, Cu and Zn accumulation in soft tissues

Throughout the current study, oyster samples predominately accumulated Cu, Fe and Zn over the rest of the elements, reflecting the metal richness previously observed for this bivalve species in other studies (e.g., Burioli et al. 2017; Cao and Wang 2016). The Pacific oyster has already been highlighted as an organism with high ability to concentrate Cu and Zn in its tissues (e.g., Funes et al. 2006; Lin and Hsieh 1999; Lu et al. 2017; Luo et al. 2014; Rojas de Astudillo et al. 2005). In fact, it is generally agreed that the highest Zn concentrations in marine biota are found in tissues of filter-feeding mollusks, especially oysters (Eisler 2000). As an example, Rebelo et al. (2003) indicated that Zn concentrations were 50 times higher in the soft tissues of the oyster *Crassostrea rhizophorae* compared with the sediment of the same study area. In the case of Cu, oysters have hemocyanin as an oxygen carrier in their blood, which might account for the much higher levels of Cu in their bodies than in other

Table 3 Metal concentrations found in the soft tissues of *Crassostrea gigas* from the present study and from other polluted marine environments from around the world (µg/g, wet weight) and National and international guidelines with maximum values allowed for metal concentrations in bivalve mollusks (µg/g)

Coastal environment/guidelines	Cd	Cu	Cr	Fe	Mn	Ni	Pb	Zn	References
Bahía Blanca estuary (Argentina)	0.29–0.89	19–81	0.042–0.42	12–230	1.5–15	0.020–0.24	< MDL	39–320	This study
Adriatic and Tyrrhenian Sea (Italy)	0.065–1.1	4.4–1200	0.0020–1.1	30–600	0.1–82	0.12–0.77	0.15–0.73	170–1000	Burioli et al. (2017)
Mailiao Industrial Harbor (Taiwan)	0.036–0.068	55–250	0.12–0.16	–	–	0.076–8.2	0.036–0.062	64–84	Fang and Dai (2017)
California coast (Mexico)	4.1–21	26–85	0.33–110	–	–	1.0–42	< MDL–4.7	95–415	Jonathan et al. (2017)
El Jadida coast (Morocco)	0.13–1.9	0.73–8.4	0.24–3.1	–	1.1–7.8	1.2–7.5	0.24–1.5	5.6–130	Maanan (2008)
FAO/WHO[a] (Europe)	1	10						150	FAO, WHO (1984)
FSANZ[b] (Australia)	2	3					2	130	FSANZ (2005)
FDA[c] (USA)	4		13			80	1.7		FDA (1993)
EEC[d] (Europe)	2						3		EEC (1995)
CAA[e] (Argentina)	2	10					1.5	100	CAA (2017)
SENASA[f] (Argentina)	1						1.5		SENASA (2017)

Data are given as range data (minimum–maximum). There are no guidelines for Fe and Mn concentrations in bivalve mollusk tissues. < MDL: below the method detection limit

[a] Food and Agriculture Organization of the United Nations/World Health Organization

[b] Food Standards Australia New Zealand

[c] Food and Drug Administration

[d] European Economic Community

[e] Código Alimentario Argentino

[f] Servicio Nacional de Sanidad y Calidad Agroalimentaria

invertebrates (Wang et al. 2011). The Fe concentrations are probably related to the Fe contents accumulated in the soils and suspended particulate fraction of the BBE, higher at several orders of magnitude compared with the rest of the metals under evaluation (La Colla et al. 2015; Serra et al. 2017).

Metal concentrations found in bivalves differ due to a species-specific ability to regulate or accumulate those elements (Yesudhason et al. 2013). Even living in the same habitat, aquatic invertebrates take up and accumulate trace metals showing variability according to the element and regarding the invertebrate taxa (Rainbow 2002). Three different metal homeostasis mechanisms have been identified in marine invertebrate cells: (1) binding to metallothioneins, (2) compartmentalization within lysosomes and (3) formation of insoluble precipitates such as Ca/Mg concretions or Ca/S granules (Viarengo and Nott 1993). In the case of Cu, Fe and Zn, granules containing these metals have already been observed in the wall of the heart of *Crassostrea* sp. (Funes et al. 2006).

The maximum values registered for Cu and Zn in soft tissues of *C. gigas* from the present research were higher only in some occasions compared with the metal values found in the same oyster species but from other coastal systems (Fang and Dai 2017; Maanan 2008) (Table 3). These results might be a consequence not only of the pollution of the aquatic environments, but also of the physicochemical factors of the aquatic systems such as salinity, dissolved organics, pH, hardness, sediment texture, factors that influence the chemical forms of metals which are also responsible for the metal bioavailability and its incorporation into the organism (Rainbow 1995).

As previously mentioned, oysters are exposed to solid and particulate matter stored in contaminated sediments (Burioli et al. 2017). Then, it becomes evident that oysters incorporate metals from the resuspended sediments, plankton and the seawater fractions and that a fraction of the particle-bound and dissolved metals is available to filter-feeding species. Having said that, metal levels in oyster tissues were in agreement with the contemporaneous metal studies done in the coastal environment of the BBE (La Colla, 2016; La Colla et al. 2015). These studies achieved, at times, both dissolved and particulate Cu and Zn concentrations above the permissible levels set by national and international guidelines.

As for the metal sources, Cu could be considered a port-related contaminant, as it is a common component in anti-biofouling paints applied on the surfaces of ships and in offshore engineering (Delucchi et al. 2008; Pan and Wang 2012). Zn is usually associated with Cu in order to improve the anti-biofouling paints efficiency (Guardiola et al. 2012). Moreover, the release of domestic wastewaters with a variety of household products might be also responsible for the concentration of Zn in the aquatic environment. The runoff from adjacent agricultural areas could be another source for Zn since it is often present in fertilizers and/or pesticides (Alloway 2013). As for Fe, sewage sludge, which affects BBE, usually contains high amounts of Fe oxides (Parkpain et al. 2000).

Cd, Cr, Mn, Ni and Pb accumulation in soft tissues

The metals Cr, Mn and Ni are also essential trace elements for living organisms, whereas Cd and Pb are nonessential metals that could be really harmful to organisms, even in trace amounts. In the present research, Cd, Cr, Mn and Ni were detected in almost all oyster tissues from within the BBE and the coastal area nearby, indicating the presence of the bioavailable metal form, but not a clear pattern was evident or high concentrations were achieved. The metals Cd, Cr and Ni achieved maximum values all below 1 µg/g (w.w.). On the contrary, all Pb values recorded were below the MDL.

The metal accumulation in oyster tissues provides a relative measure of the total metal intake by an organism, integrated over a preceding time period (Dos Santos et al. 2010), and differences exist in the form of accumulation between essential and nonessential metals. The accumulation of nonessential metals like Cd and Pb involves the induction of metallothioneins as reported by Bebianno and Langston (1995) and Bebianno and Serafim (2003). Metallothioneins are metalloproteins involved in metal detoxification and recognized as a biomarker of defense (Haidari et al. 2013). In the case of Cd, the metal uptake by marine organisms depends on its chemical speciation, being Cd^{2+} the more bioavailable species, and influenced mainly by water salinity and by the presence of organic ligands in water (Burioli et al. 2017). The Cd concentrations achieved in this study were all below the national and international guidelines for oyster consumption, while, on the other hand, dissolved Cd values reported in contemporaneous studies done in the BBE achieved Cd values that were, at times, above the limits set by international guidelines for marine coastal environments (La Colla et al. 2015). The fact that Cd values in one matrix are beyond the permitted limits while in the other are considered to be low could be explained by the metal incorporation and accumulation pattern in living organisms. This pattern depends not only on the concentration of the element in water but also on the ontogeny and environmental parameters involved (e.g., salinity, pH, water hardness) (Al-Weher 2008).

As for Pb, undetectable concentrations like the ones in this research are usually found in shellfish because of the limited bioavailability of both dissolved and dietary Pb to marine animals (Burioli et al. 2017). Pb exhibits a high affinity toward organic substances and clay minerals, resulting in a preponderance of the sediment-bound form. In previous researches done in the BBE, Pb values were close to the limit of detection in the majority of the sampling sites, in both the dissolved and particulate water fractions (La Colla 2016). The introduction of Pb-free petrol during the previous decades might account for the low current concentrations found in the oyster tissues.

In the present study, Ni values were all below 1 μg/g and the concentrations achieved could be mainly linked to the suspended sediment material and the food, since contemporaneous studies on Ni in the dissolved seawater fraction were all below MDL (La Colla 2016). Indeed, other studies have already proved oysters to efficiently assimilate Ni ingested with the food (Zaroogian and Johnson 1984, and references therein).

Bivalves also have the ability to concentrate elements like Cd, Ni and Pb in their shells (Edward et al. 2009). Metal accumulation in the shell of the gastropods could be due through the substitution of the calcium ions in the crystalline phase of the shell or due to their association with the organic matrix of the shell (Edward et al. 2009). Then, this would be an interesting subject for further studies.

In the case of Cr, marine shellfish appear to have a low tendency for Cr bioaccumulation (Chong and Wang 2001; Rojas de Astudillo et al. 2005). This information is in good agreement with the low Cr values found in the present research. The physiology of the oysters allows them to greatly discriminate against Pb and Cr and toward essential metals like Cu and Zn (Huanxin et al. 2000).

As for Mn, this metal is believed to be one of the least toxic, and only a few published reports exist on Mn toxicity in marine organisms (Martin et al. 2008 and references therein). Oyster species incorporate Mn during biomineralization in their hard part (i.e., the shell and the ligament), depending primarily on its concentration in the water. Studies on Mn partitioning between the soft tissue and shell state that the mantle tissue takes up Mn directly from seawater for incorporation into the shell, with no involvement of the remaining soft tissue (Barbin et al. 2008). Thus, it is not strange to find low concentration in the soft tissues of *C. gigas.*

Concentrations found for Cd, Cr, Mn, Ni and Pb in oyster tissues were compared to those found in other researches from worldwide coastal environments (Table 3). Except for Pb, metals showed concentrations that were usually within the same order of magnitude, or even lower, than the metal concentrations reported in other studies. As for Pb, concentrations achieved throughout this study were much lower than values registered in other coastal areas. The lack of previous data on metal values in oysters from the BBE restrains the possibility of a detailed assessment of the contamination trends in the Bahía Blanca estuarine and coastal area.

As for the sources, Cd could be particularly found in the waters as a consequence of the phosphate fertilizers from the adjoining areas (Jonathan et al. 2017). Cr is an important component of stainless steel and is also used for production of pigments, tanned leather, anticorrosive and wood preservatives (Das and Mishra 2008). The main sources of Cr to the marine environment are wastes of metal finishing industries, dumping of solid wastes and municipal wastes, among others (Dahab and Al-Madfa 1997). Also, it is possible to relate Cr values to atmosphere deposition (Rosas et al. 1989). Ni is used in several industrial applications such as in electroplating, storage batteries, automobiles, aircraft parts, spark, electrodes, cooking utensils, pigments, lacquer cosmetics, water and printing fabrics (Rahman et al. 2012). Ni is an important indicator of oil pollution, as it is part of crude oils, generally in higher concentrations than other metals (Haidari et al. 2013). Pb is a toxic metal of concern to human health, and its distribution is mainly related to the tetraethyl lead gasoline. Other sources involve industrial particulate emissions, fossil fuel burning (Lantzy and Mackenzie 1979) and atmospheric dust (Gaiero et al. 2003). Like for Fe, sewage sludge usually contains high amounts of Mn oxides (Parkpain et al. 2000).

Metal distribution in the coastal ecosystem

According to the analyses of the data, the PCA and the multidimensional scaling, metal accumulation in the soft tissues of *C. gigas* varied according to the sampling sites and areas of analysis. The results showed the highest values to be estuary related, with the sampling site located outside the estuary with the lowest values. Among the eight metals, Fe and Zn had a strong contribution in discriminating the inner and outer estuarine environment. The other trace elements also managed to characterize the metal distribution, showing higher Cd, Cu and Zn values toward the outer estuary (i.e., sites 4, 5 and 6) and higher levels of Cr, Fe and Mn toward the inner zone (i.e., site 3). The reason for these results possibly lies on the impact of human activities surrounding the estuary, streams and rivers that outflow within the estuary and the coastal area nearby. The outer estuary is influenced by the port systems of Coronel Rosales and Puerto Belgrano. Thus, it is not strange to achieve the highest Zn and Cu levels as they are both port related. On the other hand, the high levels of Fe and Mn found toward the inner estuary are good indicators of the presence of sewage sludge from cities like the ones located on the margins of the BBE.

The results also indicated within-site variability, characterized by the highly heterogeneous variances and the large variations between minimum and maximum values. These differences are not unexpected anyway, because of the complex relationships between environmental concentrations and bioaccumulation (Alfonso et al. 2013). In addition, the interaction among the metals might affect their accumulation pattern. It has previously been shown that exposure to one metal possibly leads to changes in the accumulation of another metal with similar physicochemical properties (Wang et al. 2011). Thus, the possibility that the metal concentrations found in the oysters might be related to their exposure to multiple metals could not be ruled out in this study. For this end, correlation analyses were used in an attempt to identify relationships between them. Indeed, Cu, Cd and Zn, which were related elements in the outer zone of the estuary, showed a strong positive correlation, possibly indicating similar physicochemical properties and binding empathies to the same proteins in the tissues. Also a positive and strong correlation between Ni and Cr and between Ni and Zn was appreciated.

Size influence on metal accumulation

Mollusks are ideal for environmental monitoring since various species are effective "sentinel" organisms due to their sedentary nature and the ability to filtrate large amounts of water, allowing them to accumulate substances from the environment. They provide a time-integrated indication of contaminants and offer advantages as pollution amplifiers of certain chemicals (Lu et al. 2017). Both essential and nonessential metals are known to be highly accumulated by the variety of mollusks species (Gupta and Singh 2011). Within a single bivalve species, metal accumulation is the result of a variety of characteristics such as the speed of growth, age, size, sex, reproductive conditions of the mollusks, season, salinity, chemical species and interaction with other pollutants (e.g., Alfonso et al. 2013; Maanan 2008; Paez-Osuna et al. 1995).

Even though it is known that the metal contents in oysters are size dependent, it is often difficult to sample oysters of uniform size both within and across sampling sites. Thus, the size variability is a significant factor affecting the interpretation of the data and cannot be neglected. As differences in oyster sizes were advised, relationships between oyster sizes and metal concentrations were evaluated. Only a slight significant (positive) effect of size on Cu concentration was achieved, but this correlation did not translate into higher metal concentrations for the oyster samples of a particular sampling site. Likewise, in other researches no obvious correlation between soft tissues and trace metal concentrations was either achieved (Richards and Chaloupka 2008 and references therein).

According to the results from the sampling areas, the harbor BP showed the highest mean size values. Concomitantly, the majority of the metal values also registered high levels in this area. Nevertheless, as other sampling areas with smaller oyster sizes also achieved high metal values, no obvious association was achieved between oyster biological parameters and trace metal concentrations. In fact, there are previous researches showing opposing results. Cheung and Wong (1992) found that small oysters contained higher metal concentrations than the larger ones. They explained that younger bivalves exhibit faster growth rates allowing rapid turnover of cellular materials and larger surface-to-volume ratio resulting in more metal intake and incorporation into the tissues. On the contrary, other researchers found relationships between soft tissue weights and metal bioaccumulation, as larger and heavier oysters incorporated more metals than their smaller counterparts (e.g., Lee et al. 2016).

In marine bivalves, condition index (CI) is related to the reproductive cycle because during the spawning period, organisms utilize energy from glycogen stores to produce gametes, leading to lower tissue weights (Allen and Downing 1986). Mann (1979) concluded that once gametogenesis commences growth may not increase with temperatures above 12 °C; consequently, a decrease in the CI is usual (Costil et al. 2005). There are previous researches regarding the reproductive seasons of the *C. gigas* species inhabiting areas close to the BBE (period 1998–2004, in Borges 2006). These related studies showed that the CI is elevated during the period April to December and every year in January a decrease in the CI is appreciated, highlighting the fact that this is the spawning period (Borges 2006). The present study was mainly conducted from August and December, and so the spawning effect was ruled out. There is only one sampling site, site 2, which could have been affected since the samples were obtained in the post-spawning period, when there is usually a significant loss in the oysters' mass weight. According to the results, even though the mean total soft tissues weight for the site 2 was among the lowest values, no such negative correlations between weight and metal values were achieved. In fact, high concentrations of the majority of the metals were found in sampling site 2 despite being in the post-spawning period.

Comparisons with other filter-feeding organisms from the Bahía Blanca estuary

There are no previous reports regarding metal concentrations in *C. gigas* tissues from the BBE as well as for the rest of the coastal systems in Argentina. Nevertheless, the mussel *Brachidonthes rodriguezii* had been already used as a biomonitoring species in this coastal area during the period 2011–2013 (Buzzi et al. 2017; Buzzi and Marcovecchio 2018). Sample concentrations were expressed as dry weight (µg/g dw). So, in order to compare the values, results were normalized to a water content of 80% according to previous studies from Gil et al. (2006).

Brachidonthes rodriguezii accumulated metals in the decreasing order (minimum–maximum) (µg/g, w.w.): Fe (106–186) > Zn (13) > Mn (3.4–5.6) > Cu (2.6–5.4) > Cd (0.28–0.62) > Ni (0.48) > Cr (0.18) (Buzzi et al. 2017, 2018). *B. rodriguezii* showed an accumulation pattern with lower concentrations of all the metals compared with *C. gigas*, with the exception of Ni. Both *C. gigas* and *B. rodriguezii* showed Pb values below the MDL in its tissues. Between these two species, differences were found as oysters accumulate Zn in detoxified granules, while mussels excrete much of the accumulated Zn in granules from the kidney. As a consequence, oysters are strong accumulators of Zn, whereas mussels are weak net accumulators or partial regulators of Zn (Amiard et al. 2008).

Guidelines for consumption

Oysters are edible marine organisms of worldwide commercial value, and in Argentina, the amount of oysters consumed accounts for a percentage of the total consumption of fish and shellfish, with an annual production of 10 tons (Ministerio de Agroindustria 2017). Oysters harvested from the BBE and the coastal zone nearby are usually used as a local food source with a frequency rarely exceeding once a week and the amount is limited, given that 12 oysters are the average portion size (Burioli et al. 2017). Local fishermen might consume oysters in a higher proportion and with a higher risk since oysters are consumed without any further health or safety controls.

According to the guidelines set by the Argentinean food legislation and the international guidelines (see Table 3), in order to assess whether there is a risk associated with oyster consumption, Zn and Cu levels in total soft tissue of *C. gigas* were above the safety guidelines in 11% and 100% of the samples, respectively. These results strongly suggest the need for regular monitoring and control of oysters harvested for human consumption in the BBE.

Final comments

The present study is a real baseline, due to the recent bioinvasion, for the concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the whole soft tissues of the Pacific oyster (*C. gigas*) in the BBE and the open marine environment nearby.

The study expected to find differences between the metal values found in the oysters from the sampling areas surveyed as a consequence of the differences in their surrounding environment and in the oyster sizes. Indeed, the results showed the highest metal values to be estuary related, with the beach area sampling site reaching the lowest ones. Among the eight metals, Fe and Zn had a strong contribution in discriminating the inner and outer estuarine environment. They also reached the highest concentrations in the soft tissues together with Cu. On the contrary, all Pb values recorded were below the method detection limit. Of all the metals evaluated, *C. gigas* achieved the highest Zn values in its tissues.

The invasive populations of *C. gigas* also proved to be a potential risk to public health, since Zn and Cu levels in total soft tissues were above the safety guidelines in many of the samples. Moreover, in comparison with the mussel *B. rodriguezii*, the other bivalve species already evaluated in the same coastal system during approximately the same sampling period, *C. gigas* registered higher concentrations of almost all the metals. These results reinforce the advantages of oysters as “sentinels” species.

Altogether, this study reported baseline data for metal levels in soft tissues of oysters from the Bahía Blanca estuary and the coastal area nearby, but additional studies on metal concentration related to different age classes and metal accumulation in the shell matrix would be interesting subjects to incorporate in further studies.

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