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Baseline trace metals in bivalve molluscs from the Beagle Channel, Patagonia (Argentina)

Marcelo Enrique Conti · Jorge Stripeikis · Maria Grazia Finoia · Mabel Beatriz Tudino

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Abstract In order to test the aptitude of individuals of *Mytilus chilensis* as biomonitors of heavy metals pollution in seawater, several samples of this mollusk together with surrounding seawater samples were collected along 170 km of the coastal area of the Beagle Channel (Tierra del Fuego, Argentina) in 2005 and 2007. The study, performed in seven locations strategically selected, involved the determination of Cd, Cr, Cu, Ni, Pb and Zn in seawaters and mollusks by atomic absorption spectrometry (AAS) and the calculation of the respective concentration factors (CFs). Obtained data were standardized and analyzed by multivariate techniques in order to establish differences between sampling sites and periods. Obtained results will be shown and the bioaccumulation ability of *M. chilensis* will be evaluated by comparison with results obtained for *Mytilus* species in different geographical marine areas. A fully discussion on the possibility of employing the results as background levels for comparative purposes in other marine waters of the world will be provided. The possible harm derived from human consumption of these mollusks will be also assessed.

Keywords Bivalve mollusks · Seawater · Trace metals · Pollution biomonitoring · Beagle channel

M. E. Conti (✉)
Department of Management, Sapienza, University of Rome, Via del Castro Laurenziano 9, 00161 Rome, Italy
e-mail: marcelo.conti@uniroma1.it

M. E. Conti
International Academy of Environmental Sciences (IAES), Campo della Chiesa, 3, 30122 Venice, Sant'Elena, Italy

J. Stripeikis · M. B. Tudino
INQUIMAE, Departamento de Química Inorgánica, Analítica y Química Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

M. G. Finoia
Istituto Superiore per la Protezione e la Ricerca Ambientale, Via di Casalotti 300, 00166 Rome, Italy

Introduction

Biological monitoring methods have significant advantages over traditional analysis of abiotic matrices (water, sediments) since they lead to a remarkable reduction in time and costs. Moreover, the chemical analysis of tissues of organisms (i.e. mollusks) provides evidence of the integrated bioavailability of trace metals in the marine environment over time. Mollusks are among the most used organisms as biomonitors for trace metal pollution in biomonitoring programmes (Directive 2000/60/EC; Conti and Finoia 2010) as they comply all the requisites already described in the literature (Rainbow and Phillips 1993; Langston and Spence 1995; Conti et al. 2007a; Phillips 1976; Boening 1999; Saavedra et al. 2004; Deudero et al. 2009). Amongst deposit and filter feeders, the latter seem to be more suitable for reflecting metal concentrations in seawater (Newman and McIntosh 1982) as they respond only to the seawater fraction presenting a clear ecotoxicological relevance (Rainbow and Phillips 1993).

Another issue of particular concern is the use of mollusks as part of the diet of mammals, particularly humans. Since the levels of heavy metals accumulated are a function of factors such as temperature, salinity, diet, spawning, seasonal variations (Boening 1999; Conti 2008), mollusks would represent a hazard for humans or other mammals since heavy metals are a threat even at trace levels (Calabrese et al. 1985).

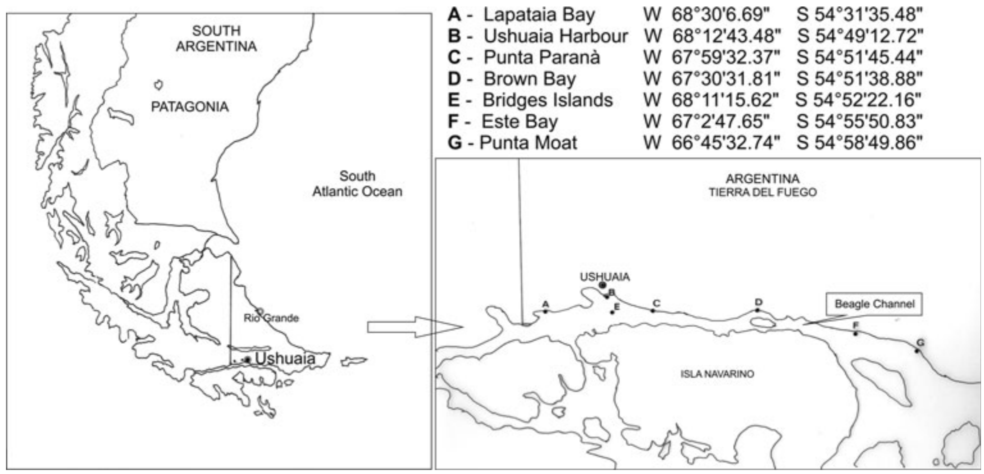

Fig. 1 The study area

According to the literature mussels filter the surrounding water (i.e. 3–9 l h^{-1} g dry $mass^{-1}$) and constantly accumulate metals in their tissues (Newell et al. 2005). Moreover they are easy to collect and to identify, both attributes which become these individuals excellent candidates for facing pollution studies.

Particularly *Mytilus chilensis* (Hupè 1854) is well distributed in South American seas (i.e. Beagle Channel, Magellan Strait, etc.) and it is also relevant from the nutritional point of view since it is a popular food in the studied areas. Consequently, it was selected to fulfill the objectives of this study: (i) to evaluate *M. chilensis* as biomonitor of pollution of Cd, Cr, Cu, Ni, Pb and Zn in the Beagle Channel, (ii) to search for strategic points in the Beagle Channel able to provide background contamination levels, (iii) to analyze the evolution of the contamination with time, (iv) to infer the daily intake of heavy metals through the consumption of these mollusks in the diet.

Results obtained by the determination of the metals listed above in mollusks and seawater samples from the Beagle Channel during 2005 and 2007 will be presented, idem with the statistical analysis. Comparison with similar studies in the literature will be provided. Findings relevant to the main objectives of the work will be shown and fully debated.

Materials and methods

Study area

Beagle Channel is a strait in Tierra del Fuego, near the southern tip of South America. The channel is about 240 km long and between 5 and 14 km wide. It separates Isla Grande de Tierra del Fuego from several smaller islands in the south. It owes its name to the British ship Beagle, employed by Charles Darwin to explore the area between 1833 and 1834. The main urban settlement in Tierra del Fuego is the city of Ushuaia that is the southernmost city of the world with ca. 60,000 inhabitants. Ushuaia is the most important port for the Antartic tourism and maritime traffic. Tierra del Fuego has a unique ecosystem and it is characterized by a wide range of wildlife and biodiversity (Conti et al. 2009; Conti et al. 2011). Except for the case of Ushuaia Harbour, the six remaining sampling sites were carefully selected as examples of supposedly unpolluted areas along the Beagle Channel.

Collection of samples

Mollusks were collected in two sampling campaigns carried out on September 2005 and September 2007. Both campaigns respected the same geographical locations. Figure 1 shows the seven selected stations situated along 170 km of the Beagle Channel.

Individuals of *M. chilensis* ($n = 280$) were collected in the tidal zone at the same depth and distance from the shoreline. Afterwards, they were put in contact ($t = 24$ h) with filtered seawater from the same site of collection for depuration purposes.

Samples were selected of about the same shell length (65 ± 5 mm) and weight in order to reduce variability due to size (Conti 2008).

Samples were placed in polyethylene bags, ice deep-frozen and transported to the laboratory. Soft parts were taken out of the shell using plastic tools (hammer and spatula) to prevent metal contamination, and then they were rinsed with deionized MilliQ water (DIW) to remove residues of shell (for sample treatment details see Conti and Cecchetti 2003; Conti et al. 2008).

Drying, mineralization and analyses of mollusks samples

Samples (400–800 mg) were previously dried, homogenized and 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 (CEM, MDS-2000) (CEM) was used for the mineralization process. The significance of the MW digestion methods is discussed elsewhere (Bocca et al. 2007). The mineralization program was run according to the manufacturer. The digested samples were made up to 25 ml with DIW.

Heavy metals were determined using atomic absorption spectrometry with graphite furnace atomization (GFAAS, Shimadzu 6800) for Cd, Cr, Ni and Pb, and flame atomic absorption spectrometry (FAAS) for Zn and Cu. Instrumental parameters and graphite furnace programs were those provided by the manufacturer. Traceability of results was obtained from the analysis of the certified reference material Antarctic krill MURST-ISS-A2 (Italian Research Program in Antarctica). The mean recovery percentages (five replicates) were: Cd: 93.4 ± 2.7%; Cr: 98.1 ± 1.0%; Cu: 101.1 ± 1.3%; Ni: 98.5 ± 2.5%; Pb: 96.5 ± 0.6% and Zn: 102.1 ± 2.9%. Detection limits (LODs) ($3\sigma b$, $n = 11$) were: Cd: 0.0001 mg l^{-1}; Cr: 0.0002 mg l^{-1}; Cu: 0.020 mg l^{-1}; Ni: 0.005 mg l^{-1}; Pb: 0.001 mg l^{-1} and Zn: 0.010 mg l^{-1}.

Samples dry weight (d.w.) (5 replicates for each location; $n = 70$ in the two sampling campaigns) was obtained by oven drying at 105°C up to constant weight.

All chemicals used throughout these experiments were ultrapure grade.

Collection of water samples and heavy metals determination

Marine water samples were collected in both sampling campaigns (7 samples each) at 2 m depth and at the same sites of mollusks collection (see Fig. 1). Duplicates were taken in each site (Süren et al. 2007; Güell et al. 2008). Salinity measurements were performed as changes in the ionic strength could affect elemental speciation (Turner et al. 2008; Conti et al. 2010)

Filtration was performed in the laboratory where water samples were passed through an acid pre-cleaned membrane filter of 0.45 μm. Afterwards, they were acidified and stored at 4°C for soluble metals determination.

For the determination of heavy metals in seawater samples, the resin Amberlite XAD-16 (Narin and Soylak 2003) impregnated with 1-(2-pyridylazo) 2-naphtol (PAN) was used for the solid phase extraction (SPE) of Cu, Pb, Cd, Ni and Zn. In this way both, the pre-concentration of the analytes as well as the isolation from the saline matrix was performed.

The solid material was slurry loaded in an acrylic microcolumn (3.0 mm i.d.; 1.8 cm long) held in a time based (T_B) flow injection manifold. The resin was retained in the microcolumn (MC) by means of two plugs of polystyrene foam placed at both ends in order to avoid leaking and/or changes in the bed volume. SPE was performed by keeping the sample at pH 9–10 by adding 2 ml of buffer NH3–NH4Cl 1 M to 100 ml of the filtrated seawater. MC was conditioned in the same way. Buffered samples were passed through the MC at an optimized flow rate of 3 ml min^{-1} during 10 min. DIW rinsing was performed between the pre-concentration cycles. Elution was performed off-line with 500 μl of HNO_3 5% m/v and the eluate was analysed for metals by GFAAS, running the programs provided by the manufacturer.

The accuracy of the analytical method was validated by the employment of the certified standard reference material Trace elements in Water (1643e, NIST). The experimental values obtained for metals concentration were in good agreement (95% confidence level) with the certified ones.

Calculus of the concentration factors (CFs)

Once heavy metals concentrations on both, mollusks and seawater samples was determined, the concentration factor was calculated as the ratio between the mean concentration of each one of the metals in the organism (Co) expressed in μg g^{-1} dry weight (d.w.) and the mean concentration in seawater (Csw) expressed in ng l^{-1}. CFs were always referred to the soluble fraction in seawater.

Statistical analysis

Different statistical approaches can be used for data analysis applied for this kind of studies (Conti et al. 2005, 2007b). Data were standardized and analyzed by multivariate techniques such as principal component analysis (PCA) and linear discriminant analysis (LDA) on PCA factors (Conti et al. 2007b; Zhou et al. 2007; Conti et al. 2010). The latter was validated by applying Monte-Carlo test on LDA (Test of the sum of a discriminant analysis eigenvalues divided by the rank, non parametric version of the Pillai's test) (Chessel et al. 2004). A number of 999 permutations were simulated: the *p*-value was highly significant when differences between groups were detected.

PCA was applied using the mean metal concentrations in *M. chilensis* as variables in order to follow bioaccumulation patterns and detect, eventually, different contamination levels amongst sites. LDA was applied for discriminating sampling sites and sampling periods (2005 and 2007) based on *Mytilus chilensis* metal contents. Data analysis was performed by Ade4 package, program R.2.4.1 and SPSS 12.1. Description of PCA and LDA techniques are reported elsewhere (Conti and Mecozzi 2008).

Table 1 Mean metal concentrations (μg g^{-1} dry weight) in the soft tissues of *Mytilus chilensis* (mean ± SD), mean metal concentrations in the two sampling campaigns (A + B) (μg g^{-1} dry weight), mean metal concentrations in coastal seawater samples, (ng l^{-1}) (mean ± SD) (n = 7 stations) and CFs[a] $\times 10^3$

	Cd	Cr	Cu	Ni	Pb	Zn
A[b]	0.72 ± 0.47	0.40 ± 0.15	6.35 ± 2.43	0.92 ± 0.35	0.43 ± 0.38	80.6 ± 82.1
B[b]	0.85 ± 0.49	0.59 ± 0.11	5.27 ± 2.04	0.93 ± 0.31	0.39 ± 0.07	90.7 ± 34.6
Mean (A + B)	0.75 ± 0.48	0.45 ± 0.29	6.14 ± 2.04	0.92 ± 0.350	0.42 ± 0.36	83.2 ± 50.8
Seawater[c] (soluble)	<18	–	311 ± 233	<100	1176 ± 1243	768 ± 369
CFs[a] $\times 10^3$	43.0[c]	–	20.4	9.5[c]	0.37	112

[a] CF = *C*o/*C*sw, where *C*o mean concentration in the organism (μg g^{-1} d.w.) and *C*sw mean concentration in seawater (ng l^{-1}). CFs are referred to the soluble fraction of seawater. Mean salinity recorded during sampling: 33 ± 1 g NaCl/liter

[b] (A) First sampling campaign—2005 (n = 210 individuals); (B) Second sampling campaign—2007 (n = 70 individuals). Wet weight/dry ratio (n = 70): 3.54 ± 0.25

[c] CFs are here intended as a minimum possible CF value obtained for *Mytilus* samples

Results

Table 1 shows mean metal concentrations (μg g^{-1} dry weight) in the soft tissues determined in each sampling campaign of *Mytilus chilensis* (mean ± SD), mean metal concentrations in the two sampling campaigns (averaged data, see row [A + B]) (μg g^{-1} dry weight), mean metal concentrations (mean ± SD) in coastal seawater samples, (n = 7 stations, ng L^{-1}), and Concentration Factors (CFs) calculated as described above. The results displayed in the Table showed that *M. chilensis* mean metal concentrations decreased in the order: Zn > Cu > Ni > Cd > Cr > Pb. The range of concentrations for each one of the metals and for the two sampling campaigns were in the range 0.13–2.30 μg g^{-1} for Cd; 3.22–13.39 μg g^{-1} for Cu; 0.04–1.95 μg g^{-1} for Cr; 0.27–2.50 μg g^{-1} for Ni; 0.10–2.89 μg g^{-1} for Pb and 21.5–348 μg g^{-1} for Zn.

Figures 2, 3, 4 show box and whiskers plots of the metal concentrations (raw data) determined in the mollusks in the selected sites and during the two sampling campaigns. The black line is the median value, the boxes represent the first and the third quartile, whilst the whiskers are set to ±1.5 times the inter-quartile interval. These values can match the minimum and the maximum values if there are no outliers and/or extreme values. For instance, we can observe clearly higher Cd concentrations in the Lapataia Bay in 2007 than those of 2005 sampling campaign (Fig. 2). In order to have more reliable results multivariate techniques were applied.

Figure 5 shows LDA on PCA results of sampling station factors for the two sampling campaigns in *M. chilensis* metal data (2005–2007). 73.97% of the total variability is explained.

Figure 6 reports LDA on PCA factors in order to point out the metal contamination evolution between the two sampling campaigns for the studied sites along the Beagle Channel. This analysis explained 100.0% of the total variance.

According to Fig. 5 and derived from the different studies described above, we can point out the following features of the different sites: (i) Punta Paraná (site C, Fig. 1) and Punta Moat (site G, Fig. 1) present high concentrations of Cu and Cr; (ii) Bridges Islands (site E, Fig. 1) shows higher Cd concentrations and lower Pb concentrations than the other sites, (iii) Brown Bay (site D, Fig. 1) and Lapataia Bay (site A, Fig. 1) show higher concentrations of Zn and Ni than the other sites.

The main observations regarding evolution of contamination in the Beagle Channel (see Fig. 6) should be summarized as follows: (i) Cu showed a significant decrease over time; (ii) Cr and Cd showed a significant increase in the second campaign, (iii) Ni, Pb and Zn showed no significant variation over time.

Some speculations on these findings will be presented with the discussion.

Discussion

The literature accounts for several studies conducted in the Magellan Strait and in the Pacific Ocean employing *M. chilensis* as biomonitor of trace metals in seawater (Astorga España et al. 1998, 2004, 2005, 2007; Tapia et al. 2010). However, very few data are available on *M. chilensis* for the Beagle Channel (Conti et al. 2006).

Even more, the comparison of our results with others performed for *M. chilensis* becomes difficult as, differently from this work, accumulation data are presented on wet basis. Here, it is important to point out that the employ of dry weight basis seems more suitable as it can prevent the high data variability derived from habitat, life conditions and conservation of organisms after sampling. As a matter

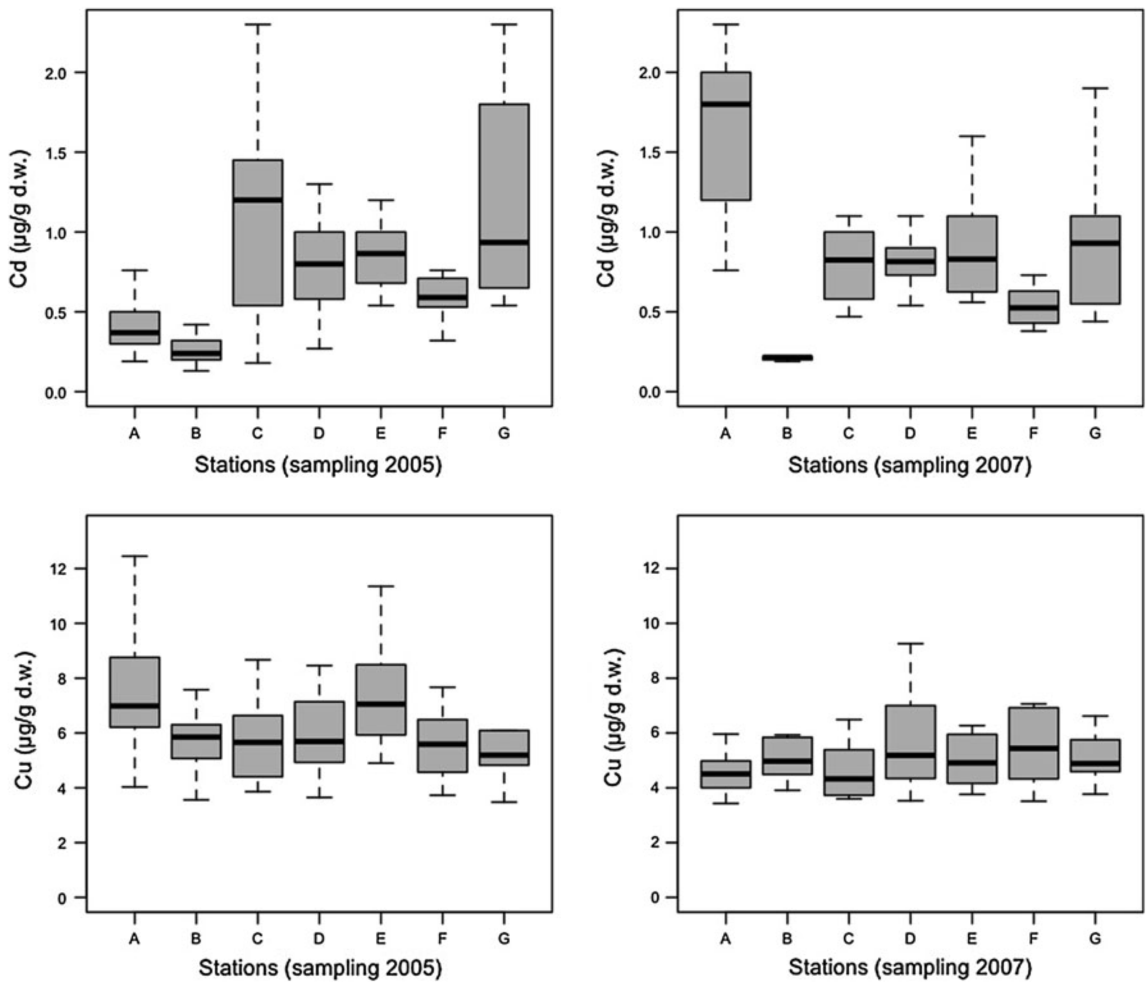

Fig. 2 Whiskers plots of Cd and Cu concentrations determined in *M. chilensis* in the selected sites and in the two sampling campaigns (2005 and 2007). See Fig. 1 for sites description

of fact, the bibliography reports ratios wet weigh/dry weight ranging between 4.32–8.50 (Crisetig et al. 1984) and 4.27–12.99 (Martinčić et al. 1992) determined in *Mytilus galloprovincialis* samples in coastal areas of the Adriatic Sea.

Taking into account the ratio wet weight/dry weight = 6.66 reported by Astorga España et al. (2004, 2005) for mussels collected in the Magellan Strait, it was possible to compare our findings with that provided by the literature. By doing this, it was observed that our mean Cd and Ni levels (0.75 and 0.92 µg g^{-1}, respectively) were lower than those obtained for the Magellan Strait (Astorga España et al. 2004). On the contrary the mean Cu and Zn levels in the Beagle Channel (6.14 and 83.2 µg g^{-1} respectively) resulted higher than those of the Magellan Strait (Astorga España et al. 2005). However, our mean Cd, Cr and Pb values matched those obtained for the Pacific Ocean (Tapia et al. 2010) (see Table 2).

The European Union (European Commission 2006) establishes safety limits for Cd, Pb and Hg in bivalves and other fishing products for human consumption. These limits, expressed as wet weight, are 1.0 and 1.5 µg g^{-1} for Cd and Pb respectively (Table 2). Surprisingly, our mean Cd levels (2.65 µg g^{-1} expressed as wet weight) in *M. Chilensis* of the Beagle Channel were higher than those established by the European Union, whilst the mean Pb level (1.49 µg g^{-1} wet weight) matched the European Union safety level (Table 2).

In relation to the intake, cadmium is a non essential element and it is toxic; its weekly intake has been established in 7 µg kg^{-1} body weight (WHO/FAO 1993). Thus, for an average adult (i.e. 65 kg) no more than 0.455 mg per week is recommended. Considering the average concentration of Cd found in Lapataia Bay in 2007 (see Fig. 2), the consumption of about 280 g (d.w.) of mollusks should be enough to reach the safety level. Nonetheless and taking into account the current consumption of mussels in the zone, this quantity is hardly attainable.

For lead, the weekly intake level is 25 µg kg^{-1} body weight (WHO/FAO 1999), which means a weekly intake

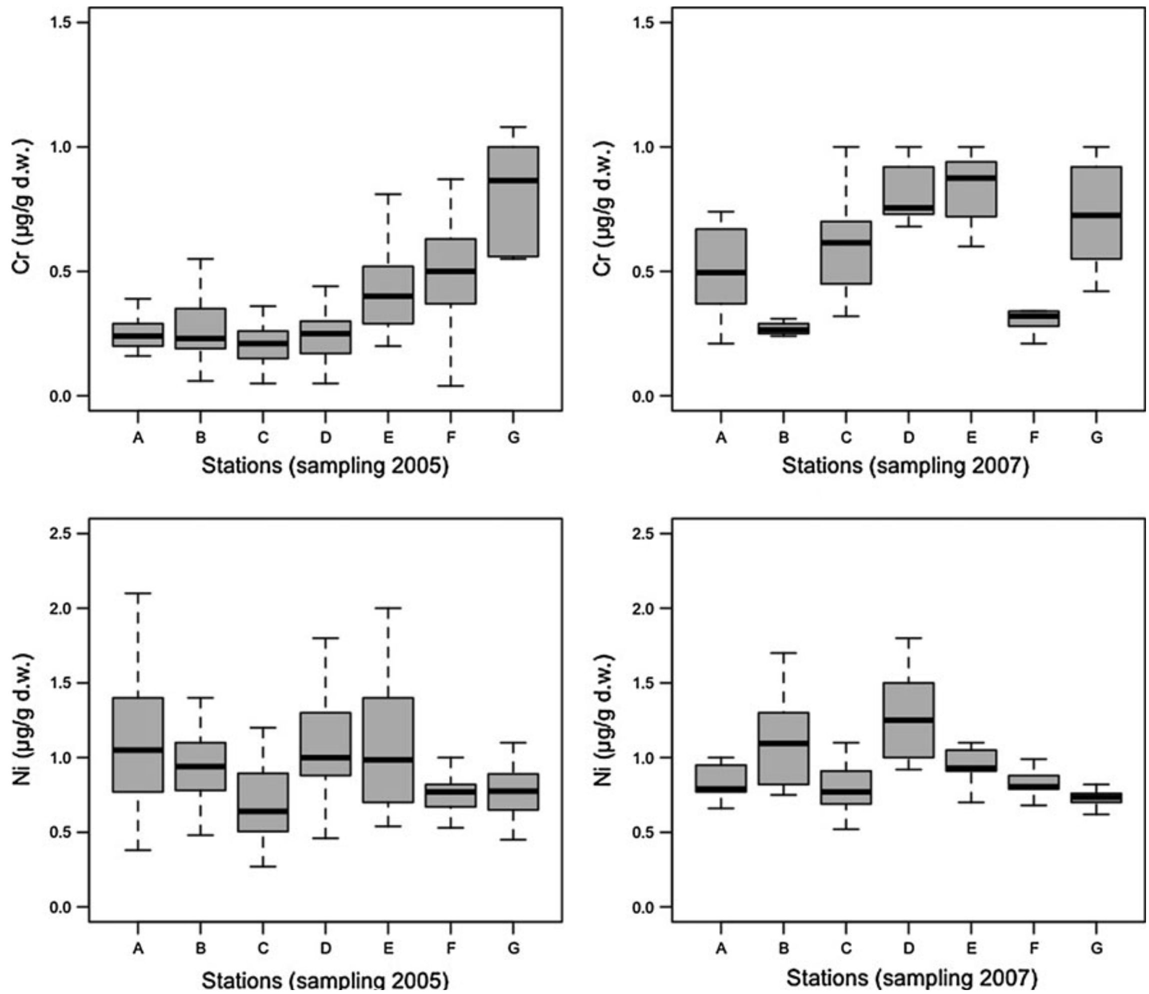

Fig. 3 Whiskers plots of Cr and Ni concentrations determined in *M. chilensis* in the selected sites and in the two sampling campaigns (2005 and 2007). See Fig. 1 for sites description

limit of 1.625 mg for an average adult. As expected, the highest Pb level (i.e. 0.76 µg g^{-1}) were obtained in the Ushuaia Harbour (2005 sampling campaign, Fig. 4). By considering this value, it would be necessary to consume about 2138 g d. w. in order to reach the WHO/FAO limit (1999). At the present and regarding Cd and Pb, it is possible to exclude risks for health of bivalve consumers.

Moreover, the US Food and Drug Administration has established tolerance levels for the toxic metals Cd, Cr and Pb in shellfish (USFDA 1993a, b, c). Considering the threshold safety limits proposed by USFDA, the levels of Cd, Cr and Pb in *M. chilensis* presented in this work resulted to be much lower than those of USFDA (see Table 2). In summary, Cd levels resulted higher than those established by the European Union, but lower than those of the USFDA.

Copper and zinc are nutrients. Copper is essential for the development of connective tissue, nerve coverings, and skin pigment. The Recommended Dietary Allowances (RDAs) for copper is 0.90 mg/day for both men and women (Food and Nutrition Board 2001). Considering that the mean Cu levels obtained for the Beagle Channel were 21.7 µg g^{-1} wet weight, we can assume that eating a normal portion of 50 g (fresh weight) of mollusks implies an intake of 1.08 mg of Cu that matches the RDAs.

Zinc is related with more than 100 specific enzymes and has high relevance in protein function and gene expression. The Recommended Daily Intake (RDI) (FAO/WHO 2002) for Zn in the adult population with moderate bioavailability of Zn in the diet is 7.0 and 4.9 mg/day for men and women respectively. Considering that the mean Zn levels obtained in this work were 294.5 µg g^{-1} wet weight, we can deduce that eating 50 g (fresh weight) of mollusks implies an intake of 14.7 mg of Zn that is much higher than the RDI level established by FAO/WHO.

Chromium stimulates insulin action in the body but it was not possible to establish the daily intake for chromium because there is a lack of information about the dose

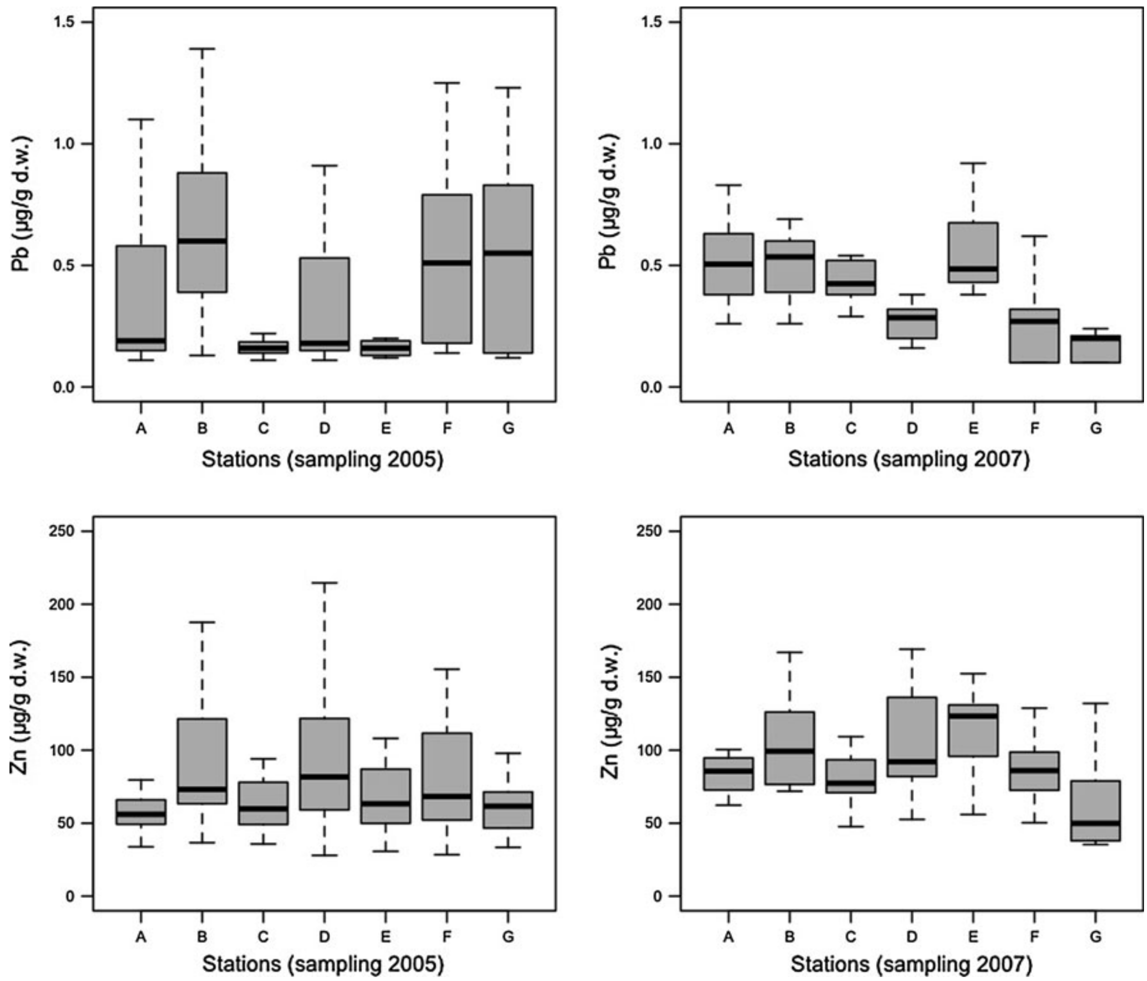

Fig. 4 Whiskers plots of Pb and Zn concentrations determined in *M. chilensis* in the selected sites and in the two sampling campaigns (2005 and 2007). See Fig. 1 for sites description

response relationship between Cr and insulin response (Food and Nutrition Board 2001). As above mentioned, the USFDA fixed tolerance levels at 13.0 µg g^{-1} dry weight. Mean total Cr levels (0.45 µg g^{-1} d.w.) found in this work were much lower than the threshold limit.

Dietary nickel is apparently non toxic to man, even cancer of the respiratory tract and dermatitis were observed in nickel refineries' workers (Reilly 1991). At present, based on adverse effects noted in animal studies, tolerable Upper Intake Levels (UILs) were set at 1 milligram per day (Food and Nutrition Board 2001). Since the mean Ni levels in *M. chilensis* found here were 3.26 µg g^{-1} wet weight, it can be said that the contribution of bivalves to Ni intake is quite low for a 50 g consumption.

Based on thousands of data arising from World Mussel Watch data sets, Cantillo (1998) proposed the metal tolerable limits in mussels that should be indicative of contamination.

Comparing our data with those limits (Table 2) we can observe that *M. chilensis* mean metal concentrations in the Beagle Channel are much lower than those proposed by Cantillo (1998). This supports the hypothesis of the Beagle Channel as background reference marine ecosystem.

Table 2 shows the comparison between the data for *M. chilensis* in the Beagle Channel obtained here with those for other *Mytilus* species in different geographical marine areas with various contamination levels. In particular, the data in this study were compared to those obtained for *Mytilus galloprovincialis* collected in the Tyrrhenian coastal areas (Italy) which were classified with low-medium level of contamination (Conti and Cecchetti 2003). The results presented in this study for Cr, Cu, Ni, Pb and Zn are clearly lower than those of the Tyrrhenian areas. On the contrary, mean Cd levels were higher in *M. chilensis* (0.75 µg g^{-1} dry weight) if compared with those obtained in *Mytilus galloprovincialis* from Tyrrhenian areas (0.38 µg g^{-1} dry

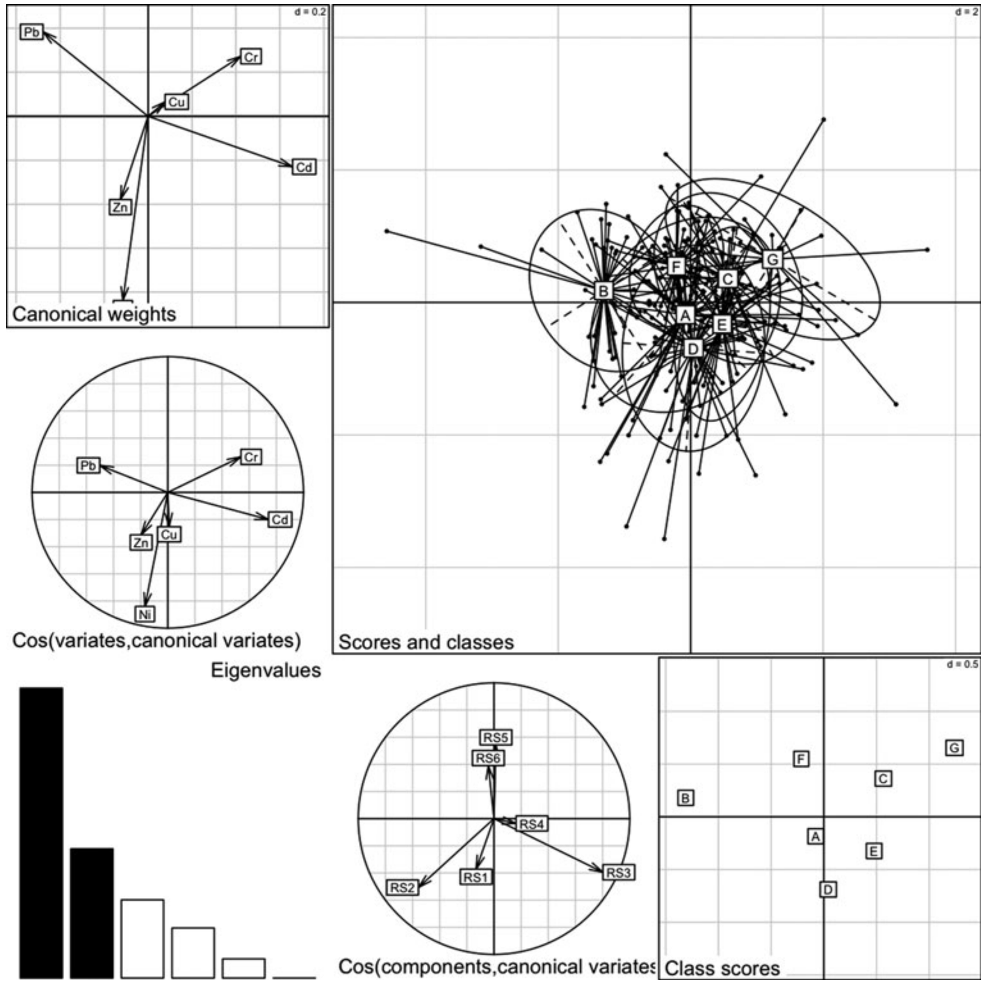

Fig. 5 LDA on PCA results of sampling station factors for *M. chilensis* metal data. This is a composed plot: [top left]: the plot of the canonical weights; [middle left] the plot of canonical correlations between variates and the first two canonical discriminant functions; [bottom left]; the eigenvalues bar chart; [bottom center]: the plot of PCA factors into LDA plane; [bottom right]: the gravity centers of classes; [main graph]: the projection of the canonical scores with ellipses and gravity center of classes. The 73.97% of total variability is explained

weight) (Conti and Cecchetti 2003). However, Cd levels in the mussels from the Beagle Channel resulted to be lower than those of other more contaminated areas such as the Moroccan Coast (Maanan 2007), the Marmara Sea (Özden et al. 2010) or the Black Sea (Çevik et al. 2008).

The mean higher levels of Cd in mollusks of the Beagle Channel could be explained by a higher bioavailability which yields to a higher accumulation. This higher biovailability could be ascribed to the chemical environment which could leave a higher amount of bioavailable cadmium in seawater (Muse et al. 2006). Since total soluble cadmium resulted non detectable with our analytical methodology, this hypothesis needs further investigation. Another possible explanation could be the presence of some concomitant able to trigger Cd accumulation, this hypothesis needs again further investigation. Nonetheless, our results (Fig. 5) show different accumulation levels depending on the sampling site which could be attributed to different speciation of Cd in each site (Muse et al. 2006). Regarding evolution with time, accumulation in Lapataia looks clearly increased in 2007 (Figs. 2 and 6) and again, a different speciation and/or an increment on the concentration of some synergic concomitant could be responsible. Maybe the spatial and temporal differences should not be attributed to differences on cadmium input in seawater but to the contribution of certain complexing chemicals able to change speciation and thus, the ability of *Mytilus* to accumulate it.

The mean Cr levels found in this survey were lower than those of other geographical contaminated areas such as the Moroccan Coast (Maanan 2007; Kaimoussi et al. 2001), Marmara Sea (Özden et al. 2010), the Black Sea (Çevik et al. 2008) and Murano Island (Venice Lagoon) (Giusti and Zhang 2002).

For Cu, the results here obtained were lower than those for *M. galloprovincialis* collected in the Moroccan Coast (Kaimoussi et al. 2001), Black Sea (Çevik et al. 2008), and Murano Island (Giusti and Zhang 2002) and matched those of obtained in Safi coastal waters, Morocco (Maanan 2007) and Korean Sea (Szefer et al. 2004).

Our results for Zn were clearly lower than those reported for *M. galloprovincialis* in several other geographical areas such as the Moroccan Coast (Maanan 2007; Kaimoussi et al. 2001), Marmara Sea (Özden et al. 2010), Black Sea

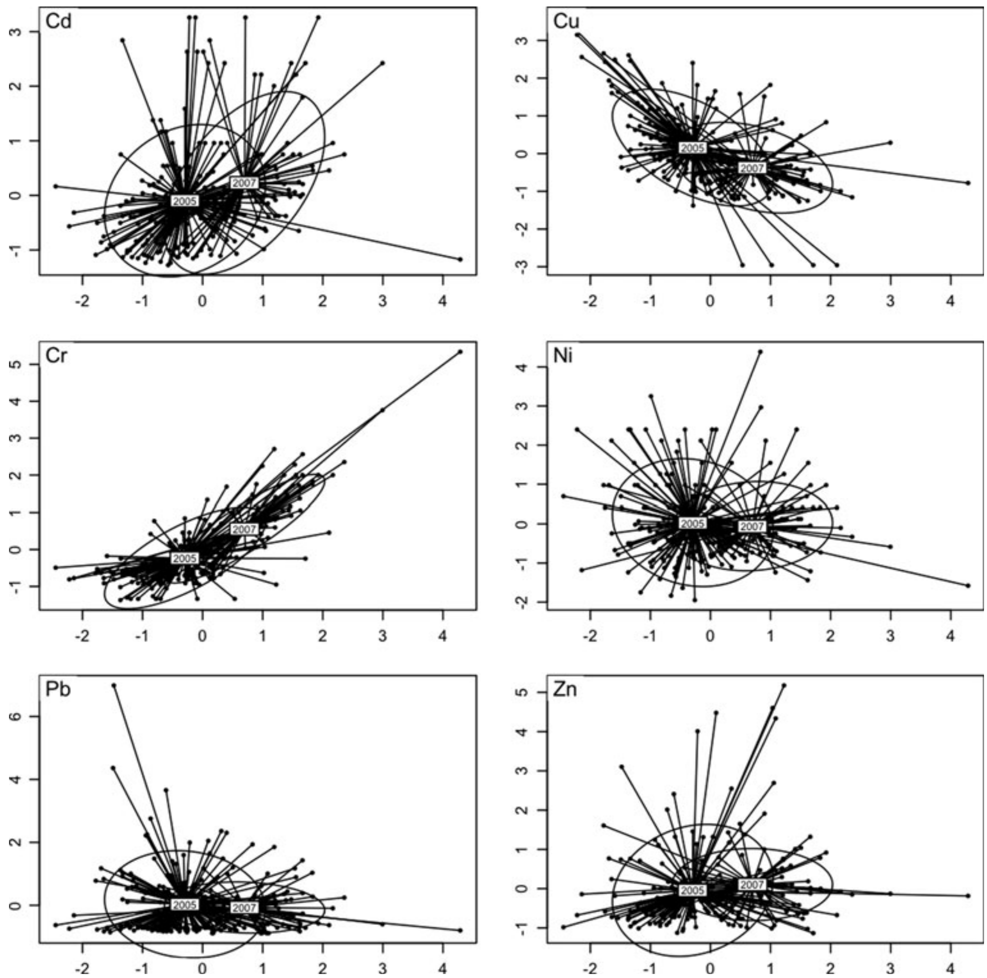

Fig. 6 LDA on PCA factors: plot of canonical discriminant scores (X-axes) and PCA scores (Y-axes). The evolution of metal concentration in *M. chilensis* samples (2005–2007)

(Çevik et al. 2008) and Murano Island (Venice Lagoon) (Giusti and Zhang 2002). Likewise, our Pb levels resulted to be lower than those obtained in other geographical marine areas such as Marmara Sea (Özden et al. 2010), Black Sea (Çevik et al. 2008), Korean Sea (Szefer et al. 2004) and Murano Island (Venice Lagoon) (Giusti and Zhang 2002). For Ni our mean level (0.92 $\mu g\ g^{-1}$) in samples collected in the Beagle Channel were lower than those collected in the Black Sea (Çevik et al. 2008), Moroccan Sea (Maanan 2007) and Murano Island (Venice Lagoon) (Giusti and Zhang 2002). Table 2 shows general data for comparison.

In general, the levels detected in *M. chilensis* in the Beagle Channel (along 170 km of coast) rank generally quite low (with the exception of Cd) compared to *M. galloprovincialis* samples arising from other low-medium contaminated and evidently contaminated areas in the world. Additionally, our results for Cd, Cr and Pb matched those obtained for Tapia et al. (2010) for *M. chilensis* sampled in the Pacific Ocean.

Seawater analysis of all the metals in solution was conducted in order to calculate the concentration factors (CFs) and to test the capability of *M. chilensis* as metal bioaccumulator (Table 1). As previously reported (Conti et al. 2010) the metal concentrations in seawater depend on many conditions such as physicochemical parameters, sampling conditions, salinity, humic substances, etc.; and this is why they have to be carefully interpreted (Turner et al. 2008; Güell et al. 2008).

Mean soluble metal levels in the Beagle Channel seawaters were low (Table 1), in particular <18 and 1176 ± 1243 ng l^{-1} for Cd and Pb respectively. These levels are well below than those proposed by the World Health Organization (WHO) and the U.S. Environmental Protection Agency since the maximum tolerable levels, that are 10 and 50 $\mu g\ l^{-1}$ for Cd and Pb respectively (Süren et al. 2007).

Comparing Cd, Cu and Zn levels in the Beagle Channel seawater (soluble fraction) with other studies, they resulted to be clearly lower than those of medium–low contaminated and uncontaminated sites in Tyrrhenian areas (Campanella et al. 2001; Conti and Cecchetti 2003; Conti et al. 2010). The mean Pb seawater levels in the Beagle Channel (i.e. 1176 ng l^{-1}) resulted to be lower than those

Table 2 Selected references of metal concentrations (μg g^{-1} dry weight) of molluscs from different geographical areas (mean values and ranges, values in parenthesis are given as fresh weight)

Species	Sites	Cd	Cr	Cu	Ni	Pb	Zn	References
M. chilensis	Beagle channel, Argentina	0.75 (2.65)	0.45 (1.59)	6.14 (21.73)	0.92 (3.26)	0.42 (1.49)	83.2 (294.5)	This work
Mussels	Mussel watch program	3.7	2.5	10	3.4	3.2	200	Cantillo 1998
M. chilensis	Magellan strait, Chile	0.86 (0.13)	–	–	1.78 (0.27)	–	–	Astorga España et al. 2004[a]
M. chilensis	Magellan strait, Chile	–	–	4.66 (0.70)	–	–	66.46 (9.98)	Astorga España et al. 2005[a]
M. chilensis	Pacific Ocean, Chile	0.21–3.40	0.38–2.91	–	–	0.43–13.70	–	Tapia et al. 2010
Bivalves	–	(1.0)				(1.50)		Tolerance level EC (European Commission, 2006)
Shellfish	–	4.0	13.0			1.7		Tolerance level USFDA, 1993a, b, c
M. galloprovincialis	Tyrrhenian Sea, Italy	0.38	0.90	8.38	–	2.07	152	Conti and Cecchetti, 2003
M. galloprovincialis	Marmara Sea, Turkey	1.77–5.87	6.18–73.8	3.76–18.85	<0.001–19.31	1.15–6.87	192.8–893.0	Özden et al. 2010[b]
M. galloprovincialis	Black Sea, Turkey	3	2	160	3	9	396	Çevik et al. 2008
M. galloprovincialis	Atlantic Ocean, Morocco	2.12–34.71	1.9–28.9	4.1–43.1	11.7–31.7	0.1–26.45	107.4–365.7	Maanan 2007
M. galloprovincialis	Korean Peninsula	0.06–2.36	–	3.93–13.6	–	3.62–52.7	70.3–157	Szefer et al. 2004
M. galloprovincialis	Atlantic Ocean, Morocco	3.00–19.30	–	6.62–331	–	–	98–530	Kaimoussi et al. 2001
M. galloprovincialis	Murano Island, Venice	–	2.0–2.4	15.8–35.5	1.6–2.6	2.5–3.9	100.2–241.6	Giusti and Zhang 2002

[a] F/S = 6.66 calculated from mean moisture percent declared by authors

[b] F/S = 6.05 calculated from mean moisture data reported from the authors

of Central Italy Tyrrhenian areas with medium–low levels of contamination (Conti and Cecchetti 2003), similar to a site with low contamination levels (Conti et al. 2010) and higher than those of an uncontaminated site in the southern Tyrrhenian areas (Favignana Island, Sicily, Campanella et al. 2001).

CFs were calculated as described above. Very high CFs were obtained for all the analyzed metals in *M. chilensis* (see Table 1) with respect to those obtained for *M. galloprovincialis* in Central Italy coastal areas (Conti and Cecchetti 2003). The CF values for Zn and Cu in *M. chilensis* were particularly high (112×10^3 and 20.4×10^3 respectively) confirming the great ability of *Mytilus* species to accumulate high levels of metals (Conti and Cecchetti 2003). Thus, in *M. chilensis*, Zn was the most abundant metal followed by Cu. The high concentration levels of Zn here obtained can be ascribed to the ability of bivalves to regulate Zn which may not be transferred along the food chain, thus limiting its concentration in the food chain (Cheung and Wang 2008). However, the ability of mollusks to regulate essential metals such as Cu and Zn is possible for a limited range of environmental concentrations of these metals (Phillips 1995).

Sites classification and the evolution of metal contamination

According to findings displayed in Fig. 5 that accounts for spatial distribution of the analyzed contaminants, it is very difficult to establish the direct source of these contaminants along 170 km of the Beagle Channel Coast. The determined levels are, except for cadmium, lower than those found for the other areas in the world. On the basis of our results we can say that the metal distribution among sites is not univocal and it is clear that none of these sites is more contaminated than the others (i.e. with clearly high levels of metals accumulation in *M. chilensis*). We must therefore reconsider the expected hypothesis of the Ushuaia Harbour as being the most contaminated site.

Moreover, comparison of metals distribution between sampling periods (Fig. 6) provides an additional evidence of not univocal distribution of metals contamination over time in the Beagle Channel.

A matter of interesting discussion is the metal biogeochemistry in coastal waters (Price and Morel 1990), particularly in the case of cadmium which presented some anomalous and hard to explain behavior between sites and periods. According to the literature (Valdés et al. 2006), Cd is removed from the superficial layer in the ocean by its accumulation in organisms' tissues and then, during the sedimentation process of the biota, the organic matter oxidation process yields Cd (and nutrients) to the aquatic medium. Then, they are pushed to the superficial layer by the upwelling costal currents. Van Geen and Husby (1996) proposed the upwelling coastal currents as a mechanism responsible for the regulation of Cd in coastal environment. This mechanism is also used as a marker of water circulation (Boyle 1988; Takesue et al. 2004). However and as told under discussion, further studies mainly involving functional speciation at ultratrace levels are needed in order to establish geochemical mechanisms for a bunch of metals in different marine coastal ecosystems. A study of possible concomitants able to trigger accumulation or depress it should be useful for a more integral understanding.

Conclusions

M. chilensis turned out to be very important for the study of coastal areas with very low levels of contamination attending to its ability to accumulate tens of thousand times higher levels of metals than those present in the seawater soluble fraction.

Regarding the consumption of these mollusks by humans, toxic metals do not represent a risk for health according to USFDA. Cadmium levels are higher than those established by the European Union but remain below those from USFDA. Moreover and according the average intake in the area, intoxication seems very unlikely.

In the case of essentials, copper ingestion matches perfectly well with the amount proposed by the Food and Nutrition Board, the same with the intake of Zn which is much higher than that established by FAO/WHO. This demonstrates the nutritional relevance of these mollusks. Ni intake, on the contrary, is quite low according to the Food and Nutrition Board.

Comparative studies reveal that our results, except Cd, are much lower than those corresponding to low-medium contaminated areas in the world. In the case of Cd, the levels found in mollusks of the Beagle Channel remain lower than those from clearly contaminated areas. The high Cd levels and their differences between sites and periods need additional investigation.

The analysis of evolution of contamination between sampling periods, allow us to confirm that there is not univocal distribution of metals' contamination in the Beagle Channel over time.

The main concluding remarks with respect to the area under study are: (a) the hypothesis of Ushuaia Harbour as being the most contaminated site amongst the seven sampling locations must be reconsidered and (b) apart from cadmium, the results support the hypothesis that the Beagle Channel could be a background reference ecosystem for marine areas in the world.

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References

Astorga España MS, Peña Méndez EM, Lecaros Palma O, García Montelongo FJ (1998) Heavy metals in *Mytilus chilensis* from the Strait of Magellan (Chile). Mar Pollut Bull 36:542–546

Astorga España MS, Rodríguez Rodríguez EM, Díaz Romero C (2004) Manganese, nickel, selenium and cadmium in molluscs from the Magellan Strait, Chile. Food Addit Contam 21:768–773

Astorga España MS, Rodríguez Rodríguez EM, Díaz Romero C (2005) Sodium, K, Ca, Mg, Fe, Cu and Zn concentrations in molluscs from the Magellan Strait (Chile): their contribution to dietary intake. Int J Food Sci Nutr 56:337–347

Astorga España MS, Rodríguez Rodríguez EM, Díaz Romero C (2007) Comparison of mineral and trace element concentrations in two molluscs from the Strait of Magellan (Chile). J Food Comp Anal 20:273–279

Bocca B, Conti ME, Pino A, Mattei D, Forte G, Alimonti A (2007) Simple, fast, and low-contamination microwave-assisted digestion procedures for the determination of chemical elements in biological and environmental matrices by sector field ICP-MS. Int J Environ Anal Chem 87:1111–1123

Boening DW (1999) An evaluation of bivalves as biomonitors of heavy metals pollution in marine waters. Environ Monit Assess 55:459–470

Boyle E (1988) Cadmium: chemical tracer of deepwater paleoceanography. Paleoceanography 3:471–489

Calabrese EJ, Canada AT, Sacco C (1985) Trace elements and public health. Annu Rev Publ Health 6:131–146

Campanella L, Conti ME, Cubadda F, Sucapane C (2001) Trace metals in seagrass, algae and molluscs from an uncontaminated area in the Mediterranean. Environ Pollut 111:117–126

Cantillo AY (1998) Comparison of results of Mussel Watch programs of the United States and France with Worldwide Mussel Watch studies. Mar Pollut Bull 36:712–717

Çevik U, Damla N, Kobya AI, Bulut VN, Duran C, Dalgic G, Bozaci R (2008) Assessment of metal element concentrations in mussel (*M. galloprovincialis*) in Eastern Black Sea, Turkey. J Hazard Mater 160:396–401

Chessel D, Dufour A, Thioulouse J (2004) The ADE4 package. I. One-table methods. RNews 4:5–10

Cheung MS, Wang WX (2008) Analyzing biomagnification of metals in different marine food webs using nitrogen isotopes. Mar Pollut Bull 56:2082–2105

Conti ME (ed) (2008) Biological monitoring: theory and applications. Bioindicators and biomarkers for environmental quality and human exposure assessment. The Sustainable World, 17. WIT Press, Southampton, UK

Conti ME, Cecchetti G (2003) A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. Environ Res 93:99–112

Conti ME, Finoia MG (2010) Metals in molluscs and algae: a north–south Tyrrhenian Sea baseline. J Hazard Mater 181:388–392

Conti ME, Mecozzi M (2008) Multivariate approaches to biomonitoring studies. In: Conti ME (ed) Biological monitoring: theory and applications. The Sustainable World, 17. WIT Press, Southampton, UK, pp 213–226

Conti ME, Iacobucci M, Cecchetti G (2005) A statistical approach applied to trace metal data from biomonitoring studies. Int J Environ Pollut 23:29–41

Conti ME, Stripeikis J, Iacobucci M, Cucina D, Cecchetti G, Tudino MB (2006) Trace metals in molluscs from the Beagle Channel (Argentina): a preliminary study. WIT Trans Ecol Environ 99:473–483

Conti ME, Iacobucci M, Cecchetti G (2007a) A biomonitoring study: trace metals in seagrass, algae and molluscs in a marine reference ecosystem (Southern Tyrrhenian Sea). Int J Environ Pollut 29:308–332

Conti ME, Iacobucci M, Cucina D, Mecozzi M (2007b) Multivariate statistical methods applied to biomonitoring studies. Int J Environ Pollut 29:333–343

Conti ME, Iacobucci M, Cecchetti G, Alimonti A (2008) Influence of weight on the content of heavy metals in tissues of *Mytilus galloprovincialis* (Lamarck, 1819): a forecast model. Environ Monit Assess 141:27–34

Conti ME, Pino A, Botrè F, Bocca B, Alimonti A (2009) Lichen *Usnea barbata* as biomonitor of airborne elements deposition in the Province of Tierra del Fuego (southern Patagonia, Argentina). Ecotox Environ Saf 72:1082–1089

Conti ME, Bocca B, Iacobucci M, Finoia MG, Mecozzi M, Pino A, Alimonti A (2010) Baseline trace metals in seagrass, algae and molluscs in a southern Tyrrhenian ecosystem (Linosa Island, Sicily). Arch Environ Con Tox 58:79–95

Conti ME, Finoia MG, Bocca B, Mele G, Alimonti A, Pino A (2011) Atmospheric background trace elements deposition in Tierra del Fuego region (Patagonia, Argentina), using transplanted *Usnea barbata* lichens. Environ Monit Assess. doi: 10.1007/s10661-011-1985-y

Crisetig G, Cattani O, Viviani R (1984) Metalli tossici nei mitili dell'alto e medio adriatico. Arch Vet Ital 35:10–16

Deudero S, Box A, Tejada S, Tintoré J (2009) Stable isotopes and metal contamination in caged marine mussel *Mytilus galloprovincialis*. Mar Pollut Bull 58:1025–1031

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off J L. 327 of 22.12.2000

European Commission (2006) Commission Regulation No. 1881/2006 of 19 December 2006, Off J Eur Communities L 364/5, 2006

FAO/WHO (2002) Human vitamin and mineral requirements. Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand 2002 FAO Rome, Italy

Food and Nutrition Board. The National Academies (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Available at: http://www.nap.edu/openbook.php?record_id=10026&page=44 (November, 5, 2010)

Giusti L, Zhang H (2002) Heavy metals and arsenic in sediments, mussels and marine water from Murano (Venice, Italy). Environ Geochem Health 24:47–65

Güell R, Aragay G, Fontàs C, Anticó E, Merkoçi A (2008) Sensitive and stable monitoring of lead and cadmium in seawater using screen-printed electrode and electrochemical stripping analysis. Anal Chim Acta 627:219–224

Kaimoussi A, Chafik A, Mouzdahir A, Bakkas S (2001) The impact of industrial pollution on the Jorf Lasfar coastal zone (Morocco, Atlantic Ocean): the mussel as an indicator of metal contamination. Comptes Rendus de l'Academie de Sciences—Serie IIa: Sciences de la Terre et des Planetes 333:337–341

Langston WJ, Spence SK (1995) Biological factors involved in metal concentration observed in aquatic organisms. In: Tessier ATD

(ed) Metal speciation and bioavailability in aquatic systems. Wiley, Chichester, England, pp 407–478
Maanan M (2007) Biomonitoring of heavy metals using *Mytilus galloprovincialis* in Safi coastal waters, Morocco. Environ Toxicol 22:525–531
Martinčić D, Kwokal Z, Peharec Z, Margus D, Branica M (1992) Distribution of Zn, Pb, Cd and Cu between seawater and transplanted mussels (*Mytilus galloprovincialis*). Sci Total Environ 119:211–230
Muse JO, Carducci CN, Stripeikis JD, Tudino MB, Fernández FM (2006) A link between lead and cadmium kinetic speciation in seawater and accumulation by the green alga *Ulva lactuca*. Environ Pollut 141:126–130
Narin I, Soylak M (2003) The uses of 1-(2-pyridylazo) 2-naphtol (PAN) impregnated Ambersorb 563 resin on the solid phase extraction of traces heavy metal ions and their determinations by atomic absorption spectrometry. Talanta 60:215–221
Newell CR, Pilskaln CH, Robinson SM, MacDonald BA (2005) The contribution of marine snow to the particle food supply of the benthic suspension feeder, *Mytilus edulis*. J Exp Mar Biol Ecol 321:109–124
Newman MC, McIntosh AW (1982) Influence of lead in components of a fresh water ecosystem on molluscan tissue lead concentrations. Aquatic Toxicol 2:25–29
Özden O, Ulusoy S, Erkan N (2010) Study on the behavior of the trace metal and macro minerals in *Mytilus galloprovincialis* as a bioindicator species: the case of Marmara Sea, Turkey. J Verbr Lebensm 5:407–412
Phillips DJH (1976) Common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper II. Relationship of metals in mussel to those discharged by industry. Mar Biol 38:71–80
Phillips DJH (1995) The chemistries and environmental fates of trace metals and organochlorines in aquatic ecosystems. Mar Pollut Bull 31:193–200
Price NM, Morel FMM (1990) Cadmium and cobalt substitution for zinc in a marine diatom. Nature 344:658–660
Rainbow PS, Phillips DJH (1993) Cosmopolitan biomonitors of trace metals. Mar Pollut Bull 26:593–601
Reilly C (1991) Metal contamination of food, 2nd edn. Elsevier Applied Science, London
Saavedra Y, Gonzalez A, Fernandez P, Blanco J (2004) The effect of size on trace metal levels in raft cultivated mussels (*Mytilus galloprovincialis*). Sci Total Environ 318:115–124
Süren E, Yilmaz S, Türcoglu M, Kaya S (2007) Concentrations of cadmium and lead heavy metals in Dardanelles seawater. Environ Monit Assess 125:91–98
Szefer P, Kim B-S, Kim C-K, Kim E-H, Lee C-B (2004) Distribution and coassociations of trace elements in soft tissue and byssus of *Mytilus galloprovincialis* relative to the surrounding seawater and suspended matter of the southern part of the Korean Peninsula. Environ Pollut 129:209–228
Takesue R, Van Geen A, Carriquiry J, Ortiz E, Godinez-Orta L, Granados I, Saldívar M, Ortlieb L, Escribano R, Guzmán N, Castilla J, Varas M, Salamanca M, Figueroa C (2004) Influence of coastal upwelling and El Niño-Southern Oscillation on nearshore water along Baja California and Chile: shore-based monitoring during 1997–2000. J Geophys Res 109:1–14
Tapia J, Vargas-Chacoff L, Bertrán C, Carrasco G, Torres F, Pinto R, Urzúa S, Valderrama A, Letelier L (2010) Study of the content of cadmium, chromium and lead in bivalve molluscs of the Pacific Ocean (Maule Region, Chile). Food Chem 121:666–671
Turner A, Pedroso SS, Brown MT (2008) Influence of salinity and humic substances on the uptake of trace metals by the marine macroalga, *Ulva lactuca*: experimental observations and modeling using WHAM. Mar Chem 110:176–184
USFDA, Food and Drug Administration (1993a) Guidance Document for Cadmium in Shellfish. US Department of Health and Human Services, Public Health Service (p. 44), Office of Seafood (HFS-416), 200 C Street, SW, Washington, DC. 20204, 1993a
USFDA, Food and Drug Administration (1993b) Guidance Document for Chromium in Shellfish. US Department of Health and Human Services, Public Health Service (p. 40), Office of Seafood (HFS-416), 200 C Street, SW, Washington, DC. 20204, 1993b
USFDA, Food and Drug Administration (1993c) Guidance Document for Lead in Shellfish. US Department of Health and Human Services, Public Health Service (p. 45), Office of Seafood (HFS-416), 200 C Street, SW, Washington, DC. 20204, 1993c
Valdés J, Román D, Dávila P, Ortlieb L, Guíñez M (2006) Seasonal variability of cadmium in a coastal upwelling system off northern Chile (Mejillones Bay, 23° S). Rev Chil Hist Nat 79:517–535
Van Geen A, Husby D (1996) Cadmium in the California current system: tracer of the past and present upwelling. J Geophys Res 101:3489–3507
WHO/FAO (1993) Summary report of the 41st meeting of the joint FAO/WHO expert committee on food additives (JECFA). FAO, Roma 1993
WHO/FAO (1999) Summary report of the 53rd meeting of the joint FAO/WHO expert committee on food additives (JECFA). FAO, Roma 1999
Zhou F, Guo H, Hao Z (2007) Spatial distribution of heavy metals in Hong Kong's marine sediments and their human impacts: a GIS based chemometric approach. Mar Pollut Bull 54:1372–1378