Applying the monitoring breakdown structure model to trace metal content in edible biomonitors: An eight-year survey in the Beagle Channel (southern Patagonia)

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ABSTRACT

The purpose of this paper is to investigate the trace metal content in edible biomonitors (i.e., mollusks) in the Beagle Channel (southern Patagonia) and to assess the human health risks associated with their consumption. Rationale: The monitoring breakdown structure (MBS) conceptual model was applied to four sampling campaigns (2005 → 2012) that collected 729 samples of *Mytilus chilensis* and *Nacella magellanica*. The composition of trace elements (Cd, Cr, Cu, Ni, Pb and Zn) in the mollusks was determined using graphite furnace (GFAAS) or flame atomic absorption spectrometry (FAAS). We compared the mean obtained values with the maximum levels (MLs) of each element established by international organizations. Then, based on semi-structured interviews, we calculated the estimated daily intake (EDI) of local residents and compared it with safety reference doses, i.e., the provisional tolerable daily intake (PTDI), provisional maximum tolerable daily intake (PMTDI), and tolerable daily intake (TDI), as well as the benchmark dose level lower confidence limit for Pb ($BMDL_{01}$, a reference point (RP)/point of departure (POD). Moreover, to obtain information about the potential health risks of ingesting heavy metals (HMs) through mollusk consumption, we evaluated the target hazard quotient (THQ) and the hazard index (HI). Findings: For Cd and Pb, 65% and 40% of bivalves exceeded the MLs established by the Mercado Común del Sur (Mercosur), respectively. Except for Cd in *N. magellanica* (i.e., 1.20 µg/kg/bw/day), EDI values were clearly lower than the safety reference doses. For Cr, Cu, Ni, Pb and Zn, mussels were safe for consumption and did not raise concerns for public health. Likewise, THQ values were well below one for most of the studied metals, indicating that the exposed human population is assumed to be safe. Occasional high consumers of mollusks from the most contaminated sites may be at some health risk. Originality: The food production system and the environment are complex systems; this is crucial to understand when we consider ecosystems as a food source (i.e., marine ecosystems). Here we consider edible biomonitors, that are organisms that can have a dual function. They are food, and at the same time, if properly calibrated, they can act as indicators of environmental quality. This study is the first to investigate relevant essential and non-essential trace metal content in two edible mollusks from the Beagle Channel in a long-term survey (2005 → 2012). The information variety was high; approximately thirteen thousand determinations were conducted to support the risk assessment for mollusk consumption. Other aspects connected with the health risks and the uncertainty factors related to the presence of essential and non-essential minerals in edible mollusks as well as the use of the MBS are also discussed.

1. Introduction

Fish and seafood products are a relevant and healthy part of the human diet. They can constitute a significant source of low-cost proteins, vitamins D, A and B12, and minerals (Özden & Erkan, 2011; Pastorelli et al., 2012; Peycheva, Panayotova, & Stancheva, 2016). The

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health benefits of seafood consumption are mostly linked to the effects of omega-3 polyunsaturated fatty acids (n-3 PUFAs), which have several potential cardioprotective effects along with their antithrombotic action (Marcovecchio et al., 2015). On the other hand, there is a safety concern associated with the consumption of fish and seafood products (i.e., mollusks) due to the phenomenon of bioaccumulation of some toxic elements such as heavy metals (HMs) (Zaza, de Balogh, Palmery, Pastorelli, & Stacchini, 2015). Metals from both natural and anthropogenic sources are pervasive in the environment, and humans may be exposed to them through various pathways (i.e., the food chain). Metals may be classified as essential when their biological role is required for the metabolism and survival of an organism; some metals are present at ppm concentrations (parts per million or μg/g), e.g., Ca, Mg, Na, K, Fe and Zn. The essential trace elements required in milligram and sub-milligram quantities are Cr, Cu, Ni, Mn, Co, Se, Mo and I (Zand, Christides, & Loughrill, 2015). The nonessential elements, such as As, Ba, Cd, Sn, Pb, Hg and Al, are food contaminants with cumulative properties and are thus considered potentially toxic (Barros Paiva et al., 2012, see also relevant books by Reilly, 1991; Szefer & Nriagu, 2007; De la Guardia & Garrigues, 2015).

Copper is essential for the development of connective tissue, nerve coverings, and skin pigment. Its deficiency provokes skeletal demineralization, decreasing iron levels in plasma. Zn is essential for growth and immune function and for the synthesis and activation of more than 300 enzymes as well as proteins such as insulin, vitamin A, and nucleic acids (Zand et al., 2015). The Cu/Zn ratio is of relevance because they are antagonists, i.e. they compete for binding sites that could produce an imbalance. Moreover, excess zinc can decrease Mg and Ca uptake and, by contrast, high levels of Ca in the diet can decrease Zn absorption (Carvalho, Coelho, Acevedo, & Coelho, 2015). The Cu/Zn ratio is linked with the oxidative stress independently of health status and, it seems to be a good indicator of the extent and prognosis in carcinoma of the gastrointestinal tract (Gupta, Singh, & Shukla, 2005). Nickel has several biological functions in the body. For instance, it has a significant role as a catalytic center in redox and nonredox enzymes. On the other hand, its accumulation in food and the environment could represent a severe hazard to human health. Among the recognized health-associated effects of nickel are skin allergies, lung fibrosis, and many degrees of kidney and cardiovascular system poisoning (Carvalho et al., 2015).

Of major concern are As, a metalloid, and the HMs cadmium (Cd) and lead (Pb), which are toxic even at low concentrations and are the main contributors to hazardous dietary exposure. On the other hand, the possible phenomenon of interaction among xenobiotics should be not excluded from these studies (Fiorito et al., 2019). For instance, Zhang, Chen, Qu, Adeleye, and Di (2017), by using an in vitro model with marine mussel cells, reported an additive genotoxic effect due to combined HM and polychlorinated biphenyls (PCBs) exposure.

Cadmium has been associated with cancer, specifically, lung, kidney, and prostate cancer (IARC, 2012). Furthermore, Cd can initiate the atherosclerosis process and is linked to cardiovascular disease, especially coronary heart disease (Tellez-Plaza, Jones, Dominguez-Lucas, Guallar, & Navas-Acien, 2013).

Lead can have several health effects, e.g., alterations in the hematological, nervous, renal, and reproductive systems (Needleman, 2004; Patrick, 2006a, b). Furthermore, Pb competes with calcium in the organism, thus disrupting neurotransmitter release and reducing mineral bone density (Beier et al., 2013; Ventura et al., 2018). Regarding Cr, it is important to note that there are two stable oxidation states of Cr (+3 and + 6) that are encountered in biological systems. Cr in the form of trivalent compounds is relatively nontoxic, and it is an essential nutrient, while water-soluble Cr compounds in the + 6 oxidation states are extremely toxic (Costa & Klein, 2006). However, Cr(VI) is scarcely present in food, which mainly constitutes a reductive medium, and it is prevalently reduced in the gastrointestinal tract (Chiesa et al., 2018). EFSA (European Food Safety Authority) (2014) reported that oral exposure to Cr(VI) is of low concern for the health of the European population, but it is of potential concern for high consumers of drinking or bottled water. The IARC classified trivalent Cr compounds in group 3 (not classifiable by its carcinogenicity to humans) and hexavalent Cr compounds in group 1 (IARC, 1990, 2012).

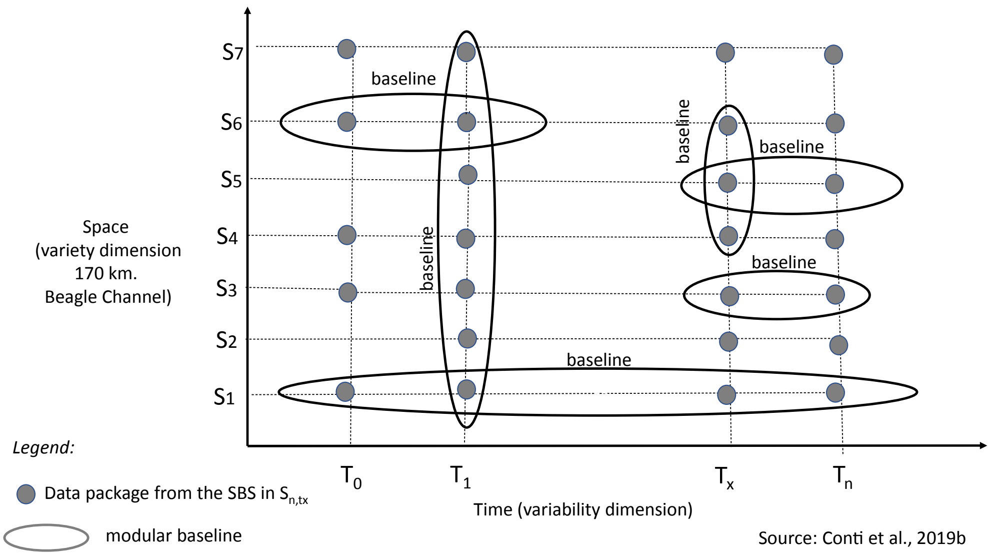
Intake studies on these elements are an appropriate tool for public health surveillance programs and involve the analysis of beneficial and harmful chemical elements in the diet (FAO/WHO, 2011), which facilitates risk assessment and the creation of certification programs for the marketing of safe food to consumers. Due to their strong ability to concentrate the metals that are present in seawater (from tens of thousands to hundreds of thousands of times), mollusks are often used as indicators of HM contamination in aquatic ecosystems (i.e., edible biomonitors) and are also an important food source (Conti & Finoia, 2010).

Several studies in different areas of the world have aimed to study the possible human health risks of mollusk consumption (Baki et al., 2018; Bonsignore et al., 2018; Connan & Tack, 2010; Crovato, Mascarello, Marcolin, Pinto, & Ravarotto, 2019; Jović & Stanković, 2014; Liu et al., 2019; Ruiz-Fernández et al., 2018; Sfriso et al., 2018; Stankovic, Jovic, Stankovic, & Katsikas, 2012; Velez, Figueira, Soares, & Freitas, 2015; Yüzereroğlu et al., 2010).

The food production system and the environment are complex systems (Conti, Canepari, Finoia, Mele, & Astolfi, 2018; Conti, Tudino, Finoia, Simone, & Stripeikis, 2019a); this is crucial to understand when we consider ecosystems as a food source (i.e., marine ecosystems). According to Ashby (1960), the understanding of a complex system depends on the information variety (requisite variety) held by the observer. In this study, we apply the monitoring breakdown structure (MBS) as a tool for the management of marine ecosystems viewed as food sources (Conti, Tudino, Finoia, Simone, & Stripeikis, 2019b). The MBS conceptual framework addresses the complexity of the food production process (mainly in its early stage) and the connected ecosystems in which food is produced. MBS takes into account the variety (space) and variability (time) dimensions. This is justified because several studies on metal intake in mollusks are based on a low quantity of samples and, sometimes, a narrow sampling period.

For this long-term survey, a supposedly uncontaminated remote geographical area was selected in order to obtain baseline data useful for future food-management actions. The accumulated trace elements can create risk for consumers, as bivalves, i.e., *Mytilus chilensis*, and patellid limpets, i.e., *Nacella magellanica*, are typical indigenous foods consumed since pre-Hispanic times by human populations along the coasts of southern Patagonia (i.e., Tierra del Fuego Province) (Pérez et al., 2017; Bigatti et al., 2018; for traceability issues also see Larraín, Díaz, Lamas, Uribe, & Araneda, 2014). For instance, *N. magellanica* has average nutritional values of 29.8% protein, 2.7% lipids and 1.8% carbohydrates (Nieto Vilela, Cumplido, Giorgis, Gil, & Bigatti, 2019). Thus, the purpose of this paper is to investigate some essential and nonessential trace metals in edible mollusks from remote areas such as the Beagle Channel (southern Patagonia, Argentina) collected from the same seven geographically referenced sites from 2005 to 2012 in four sampling campaigns.

The originality of the survey lies in the rationale according to which data have been collected, organized, and then interpreted. Consistently with the complex nature of the marine ecosystem, the paper shapes a useful framework - the monitoring breakdown structure (MBS) - that takes into account both the variety (space) and the variability (time). Variety and variability constitute two crucial dimensions of the complexity of the ecosystems, and they must be taken into account to deeply analyzing them. By applying the MBS, hence, in this work, we have on purpose increased the information variety endowment (six metals, up to 729 samples, seven sites, about thirteen thousand analytical determinations) aiming to have more reliable results about trace metals content in edible biomonitors, and aiming to helpfully supporting a sustainable management of the marine ecosystem meant as a huge, valuable, irreplaceable food provider.



Source: Conti et al., 2019b

Fig. 1. The Monitoring Breakdown Structure conceptual model: The TimeSpace Monitoring Matrix.

Thus, we have compared the mean heavy metal values obtained in the four sampling campaigns (2005–2012) with the maximum levels (MLs) established by international organizations, i.e., the Mercado Común del Sur (Mercosur); the Commission Regulation (EC) (EC) (EC), 2006; the China Food & Administration, 2017 (CFDA); and the Codex Alimentarius (Alimentarius, 2019). The US Food and Drug Administration (US FDA, 2019) reported that toxic element guidance levels for fish and fishery products for arsenic, cadmium, lead, and nickel are no longer listed.

Subsequently, based on mollusk consumption data obtained from semistructured interviews, we assessed the estimated daily intake (EDI) of the analyzed metals (i.e., Cd, Cr, Cu, Ni, Pb, and Zn) and compared them with the safe or acceptable reference dose (i.e., the provisional tolerable daily intake (PTDI), provisional maximum tolerable daily intake (PMTDI), tolerable daily intake (TDI) and benchmark dose lower confidence limit (BMDL_{01}, a reference point (RP)/point of departure (POD).

Moreover, to obtain information about the potential health risks of HM mollusk consumption, in particular for people living on the coast, we evaluated the target hazard quotient (THQ) and the hazard index (HI). THQ represents the potential noncarcinogenic risk caused by HMs ingested through oral intake (US EPA, 2019). The HI refers to possible simultaneous exposure to many potentially toxic elements given by the consumption of a particular food type (Antoine, Fung, & Grant, 2017).

Finally, the aspects connected with health risks and the uncertainty factors influencing the assessment and the presence of essential and nonessential minerals in edible mollusks as well as the use of the MBS are debated.

2. Materials and methods

2.1. *Rationale: the monitoring breakdown structure (MBS)*

As already reported, the marine ecosystem is a complex system: i.e., roughly, a system “made up of a large number of parts that interact in a nonlinear relationship” (Simon, 1962: 468). The complex nature of the marine ecosystem should not be neglected when we consider, manage and exploit it as if it were an infinite and perfectly predictable source of valuable products and services able to satisfy our several and increasing needs (energy, food, transport, etc.). Mussels provide vital ecological services such as food and habitat to a lot of other species. They are primary consumers and act as a mean for transfer of pollutants from the abiotic phase and the primary production level to the higher trophic levels (Beyer et al., 2017)

On the contrary, a focus on the complex nature of the marine ecosystem seems to be increasingly needed in the Anthropocene era in which more and more it emerges a dramatic call to linking efficiency and efficacy (imperatives of the mass production paradigm) to viable sustainability (Simone and Barile, 2015, 2016). In other words, in the Anthropocene era, supporting the safety and sustainability standards claims for more stricter rules for protecting the environment and people’s health. To this aim, it is of crucial relevance to studying and managing the marine ecosystem consistently with its intrinsically complex nature featured by variety and variability. Accordingly to this, in the present survey, we collect and organize data consistently with the variety and variability dimensions featuring the marine ecosystem complexity. The variety is a synchronous dimension, i.e. it is connected with space and, to be taken into account, it asks for different sampling sites at the same time. In the present survey, variety means that in different places at the same time different levels of essential or toxic metals in seafood can be accumulated and then detected. The variability, instead, is a diachronic dimension: it is related to the possibility of a phenomenon shows variants over time. In this survey, variability refers to a change in the metal bioaccumulation levels over time in the same area. The number of variations that the system presents at a given time and the degree of change over time is a measure of complexity (Conti et al., 2019b).

Taking into account variety and variability leads to the proposed complexity-based conceptual framework: the monitoring breakdown structure (MBS). Differently to many studies on metal intake from mollusk consumption, that are based on a low quantity of samples and, sometimes, refer to a narrow sampling period, the MBS considers keeping into account the variety (space) and variability (time) dimensions of the selected marine ecosystem (the Beagle Channel, southern Patagonia) (Fig. 1). Framing the sample consistently with the complex nature of the marine ecosystem is fundamental in order to have a satisficing (if not optimal) understanding of the ecosystem under focus. According to Ashby (1960), the understanding of a complex system (requisite variety) depends on the information variety endowment owned by the observer/decision maker. In other words, in order to understand and effectively manage a complex system, the more the complexity of the system under focus (expressed in terms of its variety and variability) increases, the more the level of the information variety (i.e. richness, abundance and diversity of the information endowment) possessed by the observer/decision maker must increase. Thanks to its consistency with the marine ecosystem complexity, in terms of variety and variability dimensions, the MBS enhances the observer's information variety endowment about the positive/negative features of the mollusks as food for human beings. Actually, being articulated along the two crucial dimensions of space and time, this conceptual framework contributes to decrease the gap between the requisite variety and the information variety endowment of the observer and leads to more helpful results in managing the marine ecosystem as a complex extraordinary food provider.

2.2. Study area and mineral analyses

Tierra del Fuego is a native ecosystem, and it has high biodiversity (Pino, Alimonti, Conti, & Bocca, 2010). The Beagle Channel (BC) is a strait in Tierra del Fuego and is considered to be of great ecological significance. It is approximately 240 km long and includes the main urban settlement in Tierra del Fuego Province, i.e., the city of Ushuaia, which has approximately 60,000 inhabitants. Ushuaia is the southernmost city in the world and is the main harbor for Antarctic tourism and maritime traffic. Aside from Ushuaia harbor, the other six sampling sites were carefully selected as apparently unpolluted areas along the Beagle Channel and possible good-quality food sources (Conti, Stripeikis, Finoia, & Tudino, 2011; Gil et al., 2019).

Thus, seven geographically referenced strategic locations along 170 km of the coastal area of the BC were selected for the four sampling campaigns, i.e., 2005, 2007, 2011, and 2012 (Fig. 2). Brown Bay (site D on the map) was selected because it is a relevant mussel cultivation area for local and tourist consumption.

The composition of trace elements was determined by using graphite furnace atomic absorption spectrometry (GFAAS) for Cd, Cr, Ni and Pb, and flame atomic absorption spectrometry (FAAS) for Cu and Zn (Conti, Stripeikis, Finoia, & Tudino, 2012).

A total of 729 samples of the bivalve *M. chilensis* and the gastropod *N. magellanica* were collected in four sampling campaigns, i.e., 2005, 2007, 2011, and 2012. Samples were collected in the tidal zone at the same depth and distance from the shoreline in the four sampling campaigns. The shell lengths and weights of the samples were kept as constant as possible in order to reduce variability due to size. Then, the samples were put in contact (24 h) with filtered seawater from the same sampling site for depuration purposes. The soft parts of the mollusk were taken out of the shell using plastic tools (hammer and spatula) to prevent metal contamination, and then they were rinsed with double deionized MilliQ water (DIW) to remove residues of the shell, placed in polyethylene bags, deep-frozen and transported to the laboratory. Sampling and chemical protocols for mollusks, including mineralization procedures, GFAAS and FAAS settings, and HM analytical determinations (figures of merit and CRM validation), have been previously reported (Conti et al., 2011, 2012).

2.3. Statistical analysis

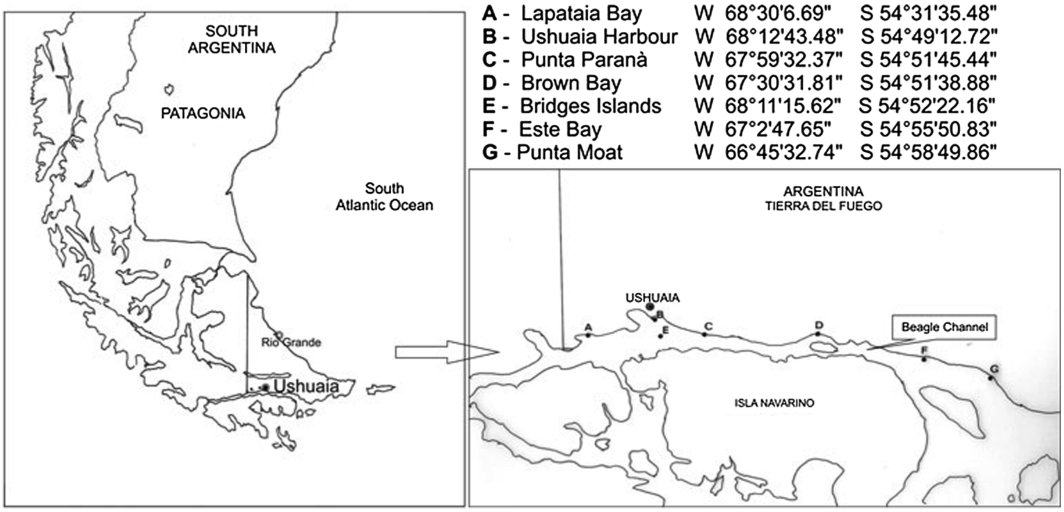
To compare the MLs for Cd and Pb for bivalve consumption established by the Mercado Común del Sur (Mercosur), box and whisker plots of the metal concentrations (raw data) measured in the mollusks from the seven selected sites through the four sampling campaigns have been reported. The black line is the median value, the boxes represent the first and the third quartile, and the whiskers are set to ± 1.5 times the interquartile interval. These values match the minimum and maximum values if there are no outliers or extreme values. This is not the case in this survey, where outliers were determined (see Figs. 3–6). To check the differences among sites, multiple median comparison tests (hereafter MMCT) were conducted (Conover, 1999; Conti et al., 2019b).

2.4. Consumption survey

Due to the lack of information as reported above, and in order to have consumption data, we conducted a local mollusk consumption survey (Dorne et al., 2011).

The first step was to identify voluntary potential eligible subjects; it should be noted that the sample was limited to the adult population only and did not include children or adolescents [Sapienza Ethical Code-D.R. n. 1636, n. 0032773, 23/05/2012].

Initially, having obtained their consent, we recruited 52 respondents. Because of incomplete data, 22 individuals were excluded, and 30 participants (adults aged 19–72) completed the whole study. We assessed bivalve and gastropod consumption by semistructured interviews by using both open-ended and closed-ended questions (Wilson, 2014). We conducted face-to-face interviews, and participants were asked about their frequency and quantity (portion sizes) of consumption of both mollusks in the past year. The baseline characteristics obtained from the respondents were age (19–72 years, mean 52 years), weight (mean 68.7 kg), height (mean 1.65 m), sex (19 men; 11 women) and educational level (from primary school to PhD). The interviews were conducted three times (once a month from September to November 2012, for testing reproducibility). The reproducibility of the declared consumption amounts was acceptable (86%). The mean weekly consumption was 50 ± 5 g for *M. chilensis* and 20 ± 4 g for *N. magellanica*.



A -	Lapataia Bay	W 68°30'6.69"	S 54°31'35.48"
B -	Ushuaia Harbour	W 68°12'43.48"	S 54°49'12.72"
C -	Punta Paranà	W 67°59'32.37"	S 54°51'45.44"
D -	Brown Bay	W 67°30'31.81"	S 54°51'38.88"
E -	Bridges Islands	W 68°11'15.62"	S 54°52'22.16"
F -	Este Bay	W 67°2'47.65"	S 54°55'50.83"
G -	Punta Moat	W 66°45'32.74"	S 54°58'49.86"

Fig. 2. The study area, Beagle Channel, Tierra del Fuego, Argentina.

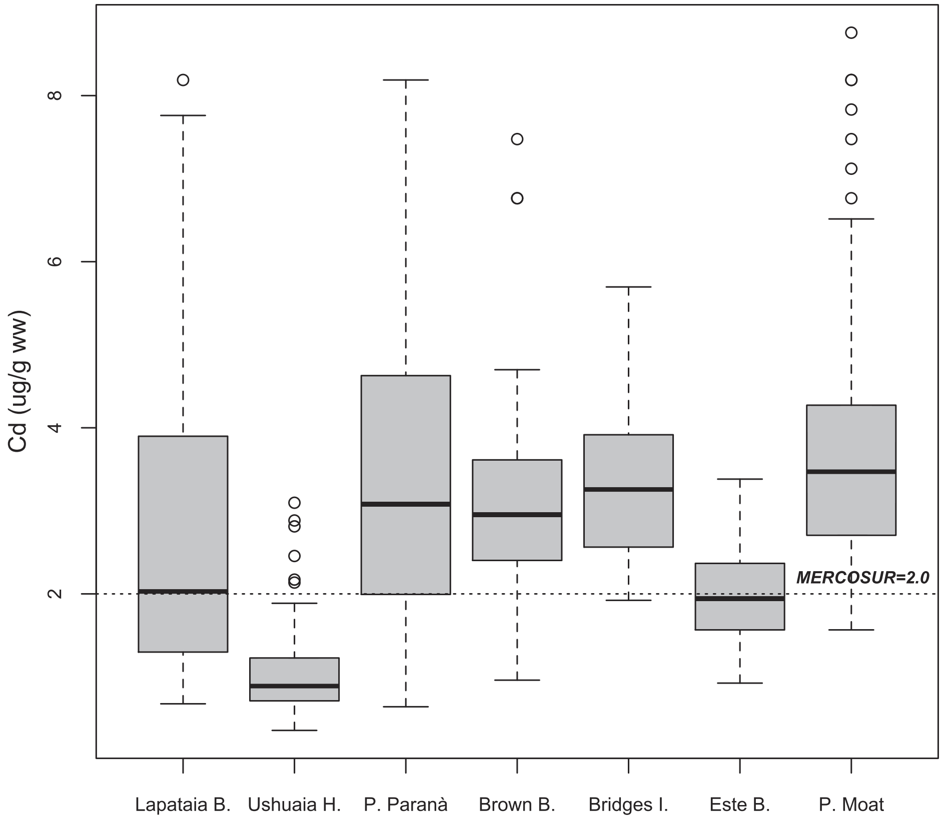


Fig. 3. Cd boxplots for *Mytilus chilensis* in the selected sites (Beagle Channel).

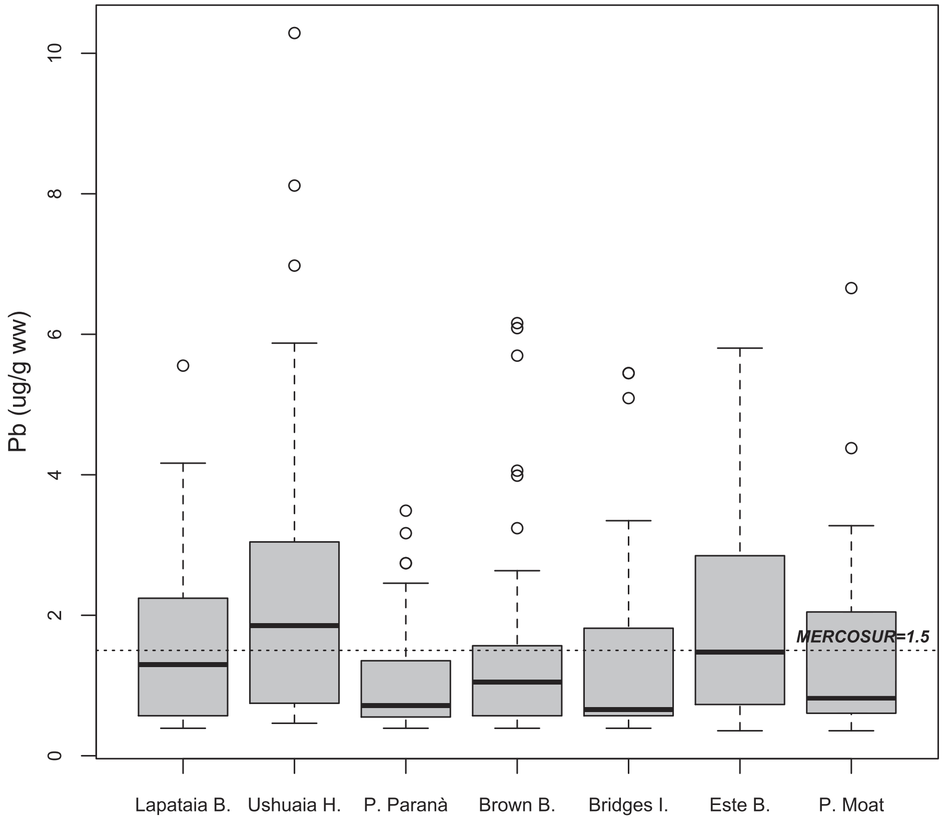


Fig. 4. Pb boxplots for *Mytilus chilensis* in the selected sites (Beagle Channel).

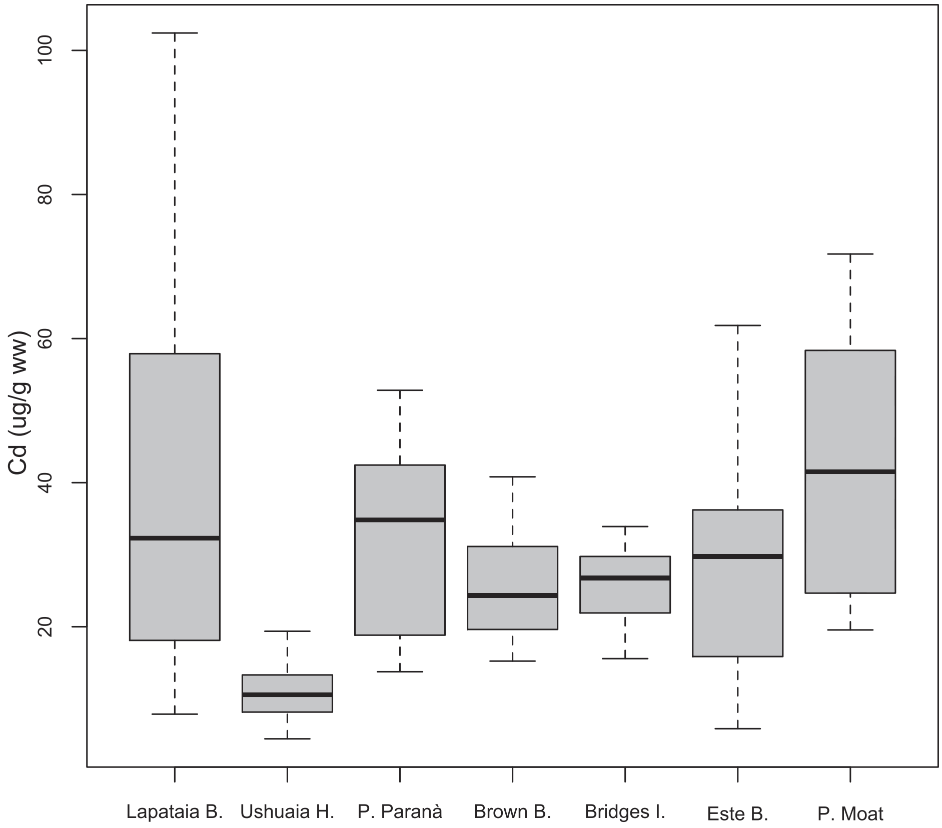


Fig. 5. Cd boxplots for *Nacella magellanica* in the selected sites (Beagle Channel).

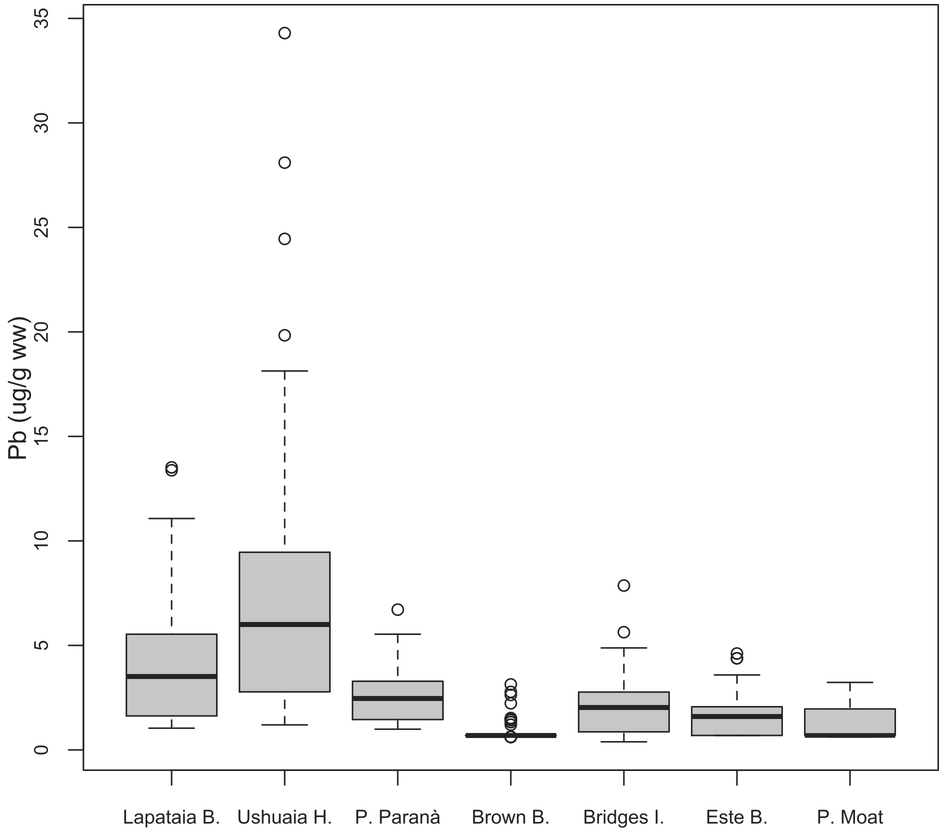


Fig. 6. Pb boxplots for *Nacella magellanica* in the selected sites (Beagle Channel).

Table 1
Descriptive data of metals concentrations in *M. chilensis and N. magellanica* samples from four sampling campaigns (2005–2012) in the Beagle Channel (μg/g wet weight)[a].

Mytilus chilensis (n=418)	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
mean	2.80	2.09	20.9	3.5	1.56	293
min	0.35	0.10	< 2.0	0.95	0.35	57.7
max	8.71	22.2	47.4	10.4	10.23	1232
SD	0.5	0.72	2.1	0.38	0.39	44.7
Nacella magellanica (n=311)						
mean	28.84	9.87	56.63	17.15	3.01	364
min	4.48	0.35	23.87	3.64	0.42	190
max	102.5	40.81	392	52.31	34.37	576
SD	2.4	1.04	6.7	1.4	0.6	9.9

[a] *M. chilensis*: wet weight/dry weight = 3.54 ± 1.01; *N. magellanica* ww/dw: 6.99 ± 1.92.

3. Results and discussion

Table 1 shows the mean, minimum and maximum metal concentrations in *M. chilensis* and *N. magellanica* in the four sampling campaigns (2005, 2007, 2011, and 2012) in the Beagle Channel. Cu was only detected in a few *M. chilensis* samples; the other metals (i.e., Cd, Cr, Ni, Pb and Zn) were detected in all analyzed samples. Our mean levels (Table 1) for the six metals determined in *M. chilensis* were clearly lower than those recently reported for mussels worldwide by Lu, Zhu, Fang, Dong, and Wang (2019). Additionally, mussel species collected in Spain showed lower levels of metals than those collected in this survey (González et al., 2019; Olmedo et al., 2013).

In contrast, the mean metal levels in *M. chilensis* (Table 1) were higher for Cd, Cr, Pb and Zn and lower for Cu than those recently measured in bivalves collected along the coast of Todos os Santos Bay, Brazil, by Barbosa et al. (2019) (i.e., 0.0180–1.69, 0.0877–1.55, 0.0675–376, 0.0612–7.09; and 10.3–46.4 mg kg^{-1} ww for Cd, Cr, Cu, Pb and Zn, respectively). A similar trend was observed comparing our higher metal ranges in *M. chilensis* with those reported for *M. edulis* collected in Sanmen Bay, "one of the most critical aquaculture bays in China" (East China Sea), i.e., 0.59, 0.43, 4.11, 0.10, and 21 μg/g ww for Cd, Cr, Cu, Pb and Zn, respectively (Liu, Liao, & Shou, 2018).

Overall, considering the length of the survey, and with some exceptions (i.e., Cd in *N. magellanica*), we can infer that metal levels in edible mollusks collected in the BC are at medium-low levels based on the literature. Thus, the concept that the BC is totally uncontaminated should be reexamined.

For the analyzed species (Table 1), we found that Zn was the most abundant metal, followed by Cu. The high Zn levels found in mollusks, as also recently reported for fishing centers in South India (Ragi, Leena, Cheriyan, & Nair, 2017), may be ascribed, in particular, to the bivalves' capacity to regulate Zn, limiting its concentration levels in the food chain (Cheung & Wang, 2008). However, the ability of mollusks to regulate essential metals (i.e., Cu and Zn) is limited to a certain range of environmental concentrations of these metals (Phillips, 1995).

Based on approximately thirteen thousand determinations of the collected samples from 2005 to 2012, the mean Cd and Pb levels in *M. chilensis* (i.e., 2.80 and 1.56 μg/g ww respectively) exceeded the MLs established by Mercosur and other international organizations (see Table 2 for comparison). It is worth noting that there are no specific metal MLs established for gastropod mollusks except for those of the CFDA.

Figs. 3–6 show the boxplots of Cd and Pb concentrations in the mollusk species and the selected sites. Fig. 3 shows that, surprisingly, the median Cd concentrations in *M. chilensis* are at the lowest levels in Ushuaia harbor (all MMCTs were significant at $p < 0.05$). This could be attributed to a different speciation of Cd at the harbor yielding species unable to be accumulated by the mollusks under study. However, considering the Mercosur limit reported in Fig. 3 (see dashed line) and Table 2 (i.e., 2.0 μg/g ww), we found that 65% of the bivalves exceeded the MLs established for Cd. For instance, the majority of the samples from Punta Paranà, Brown Bay (a mussel cultivation area), Bridge Islands and Punta Moat exceeded the limit (see the respective interquartile intervals, Fig. 3). The overall median is 2.55 μg/g ww, while the confidence interval includes values in the range 0.67–7.00 μg/g ww.

Table 2
Maximum Levels (MLs) of HMs established by international organizations for bivalves.

MLs (mg Kg^{-1} fresh weight)	Cd	Cr	Pb
Mercosur[a]	2.00		1.50
Commission Regulation (EC)[b]	1.00		1.50
CFDA[c]	2.0	2.0	1.5
Codex Alimentarius[d]	2		

[a] Mercosur, 2011.
[b] Commission Regulation (EC), 2006.
[c] CFDA-China Food and Drug Administration, 2017, the Cd limit concerns bivalves and gastropods with removed viscera.
[d] Codex Alimentarius, 1995 (amended 2019).

Fig. 4 shows that the median concentrations of Pb in *M. chilensis* were lower at Bridge Islands and higher at Ushuaia Harbor than at the other sites (MMCT significant at < 0.05). The overall median is 1.06 μg/g ww, while the confidence interval range is 0.46–4.32 μg/g ww for Pb. Considering the lead MLs for bivalves established by the Mercado Comùn del Sur (Mercosur, 2011) (Fig. 4 and Table 2), we found that 40% of bivalves exceeded the Pb MLs.

Fig. 5 shows, unexpectedly, that the median Cd concentrations in *N. magellanica* are at their lowest levels in Ushuaia harbor (similarly to *M. chilensis*), and the highest levels of Cd in *N. magellanica* were found at Punta Moat (all MMCTs were significant at $p < 0.05$). The overall median is 25.9 μg/g ww, while the confidence interval is in the range 6.98–69.6 μg/g ww. Fig. 6 shows that the median concentrations of Pb in *N. magellanica* are lower at Brown Bay and higher at Ushuaia harbor than at the other sites. Brown Bay, as reported above, is a mussel cultivation area where limpets are also naturally present and may be easily collected. Data variability was high for the Ushuaia harbor, Lapataia Bay and Bridge Islands sites (MMCTs significant at $p < 0.05$). The overall Pb median for limpets was 1.74 μg/g ww, while the confidence interval range for Pb was 0.62–6.92 μg/g ww. The lowest Cd levels determined in Ushuaia harbor for both mollusks could be attributed to a different speciation of the metal as reported above.

3.1. Estimated daily intake (EDI)

We then calculated the intake and compared the results with health-standard values with the safety or acceptable reference doses (Dorne et al., 2011; Primost, Gil, & Bigatti, 2017; USEPA, 2007; Yusà & Pardo, 2015). In the BC, mollusks (in particular bivalves) are typical indigenous foods. They are mainly an excellent, low-cost protein source for the population. However, a potential risk for human consumption may be present due to the bioaccumulation of toxic metals. This is consistent with the high concentration factors (CFs) obtained for these metals in the surveyed area. CFs are given by Co/Csw, where Co is the mean concentration in the organism (μg/g dry weight) and Csw is the mean concentration in seawater (ng/L). We determined CF values from 0.37×10^3 for Pb to 112×10^3 for Zn in Mytilus. Likewise, for Nacella, we determined 0.44×10^3 for Pb to 301×10^3 for Cd (CFs are referenced to the soluble fraction of seawater) (Conti et al., 2019b). These results confirm that these two species are strong cadmium

Table 3

Estimated daily intake (EDI) of HMs from *M. chilensis* and *N. magellanica* consumption by adults living in the coastal areas of the Beagle Channel (μg/kg bw/day). Provisional tolerable daily intake (PTDI), Provisional Maximum Tolerable Daily Intake (PMTDI), Tolerable Daily Intake (TDI) and Benchmark Dose Lower confidence Limit for Pb (BMLD$_{01}$ - Reference Point (RP)/Point of Departure (POD), are reported for comparison.

Heavy metals	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
EDIs (μg/kg bw/day)[a]						
M. chilensis (n=418)	0.30	0.22	2.25	0.38	0.17	31.56
N. magellanica (n=311)	1.20	0.40	2.35	0.71	0.12	15.40
PTDI (μg/kg/ bw/day)	0.83[b]	3.64[c]	-	-		-
PMTDI (μg/kg/ bw/day)	-	-	500	-		300-1000
TDI (μg/kg/ bw/day)	-	-	-	2.8[d]		-
BMDL$_{01}$ (μg/kg/ bw/day)					1.50[e] 0.63[f] 0.50[g]	

[a] EDIs values for an adult were calculated according from the obtained mean metal values (Table 1).

[b] PTDI values of Cd (i.e. 0.83 μg/kg bw/day) were recalculated on a daily basis from the PTMI (25 μg/kg bw/month) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2019).

[c] The Cr limit of 250 μg per day (WHO, 1996; EFSA, 2014) is divided by the mean adults weight (68.7 kg) obtained for the Beagle Channel interviewed inhabitants.

[d] EFSA (2015).

[e] Systolic Blood Pressure as endpoint.

[f] Chronic kidney disease.

[g] Neurotoxicity in young children (EFSA, 2012).

bioaccumulators (see Figs. 3, 5).

The EDI represents an estimate of the daily exposure level of the human population to HMs through food consumption (Baki et al., 2018; Liu et al., 2019).

EDIs were calculated by using the formula (Liu et al., 2019; Mok et al., 2014): F × D × I × C/W × T, where F is the exposure frequency (365 days/year); D is the exposure duration (70 years); I is the ingestion rate of *M. chilensis* and *N. magellanica* determined by the semistructured interviews (see section 2.4), which was 7.14 and 2.85 g/person/day, respectively; C is the concentration of heavy metals in the edible tissues (wet weight); W is the average body weight (68.7 kg for adults in this survey); and T is the average time (365 days/year multiplied by the number of exposure years, assuming 70 years in this study).

Table 3 shows the EDIs of HMs from *M. chilensis* and *N. magellanica* consumption by adults living in the coastal areas of the Beagle Channel (μg/kg bw/day). The provisional tolerable daily intake (PTDI), provisional maximum tolerable daily intake (PMTDI), and tolerable daily intake (TDI) as well as the benchmark dose level for Pb (BMDL$_{01}$, a reference point (RP)/point of departure (POD) are reported for comparison.

From this study, we observed that our EDI values for the two edible biomonitors were clearly lower than the safety reference dose, with the exception of Cd in *N. magellanica* (i.e., 1.20 μg/kg/ bw/day, see shaded box, Table 3). Even if the determined consumption levels were low (20 g/week), our study indicates that eating *N. magellanica* creates a certain health risk, particularly for occasional high consumers. These results agree with those of previous studies in which high levels of Cd in Patagonian edible mollusks were found (Primost et al., 2017).

In particular, regarding Pb (Table 3), based on relevant epidemiological studies, EFSA (2012) has established a range of benchmark dose level (BMDL) confidence limits to establish the 95th BMD of 1% extra risk (BMDL$_{01}$) as a reference point (RP) or point of departure (POD) for Pb risk characterization. For adults, BMDLs$_{01}$ of 1.50 μg/kg body weight per day (with effects on systolic blood pressure as the endpoint) and 0.63 μg/kg body weight per day (with chronic kidney disease as the endpoint) were estimated. For children (who were not part of this study), a BMDL$_{01}$ of 0.50 μg/kg b. w. per day was established (EFSA, 2012). On the other hand, for the other metals, i.e., Cr, Cu, Ni, Pb and Zn, if this level of intake is maintained, mussels are safe for consumption and do not raise concerns for public health. However, it is also relevant to consider that other food sources should be assessed for their impact on metal intake in the studied population.

3.2. Total hazard quotient (THQ)

THQ represents the potential noncarcinogenic risk for orally ingested HMs, and it is expressed as the ratio of the daily oral intake and the oral reference dose (Petroczi & Naughton, 2009; US EPA, 2019; Liu et al., 2019) with the following equation:

$$\mathbf{THQ = EDIs/RfDo}.$$

The THQ < 1 means the exposed population is assumed to be safe. A THQ value ranging between 1 and 5 means that the exposed population is in a concern level. It is a dimensionless index, and its values are additive but not multiplicative (Hague, Petroczi, Andrews, Barker, & Naughton, 2008; Naughton & Petróczi, 2008; Petroczi & Naughton, 2009). The reference oral dose (RfDo) represents a daily exposure dose to which humans are constantly exposed over a lifetime without having a considerable risk of carcinogenic effects (Liu et al., 2019). Due to the lack of complete estimations of RfDos for Cr, Zn and Ni, in this study, it is assumed that all Cr is Cr(VI), all Zn is Zn and Zn compounds, and all Ni is Ni soluble salts for which RfDos are available (US EPA, 2019).

Regarding cancer risk, oral intake of arsenic with food has been strongly linked to lung, bladder and skin cancer (Chiesa et al., 2018; EFSA, 2009a). Like with bladder cancer for Cd, lung cancer is a toxicological effect of Cd and Ni but only through inhalation exposure (EFSA, 2009a,2009b, 2015). Thus, here we have only considered the noncarcinogenic effects of HMs because cancer risk due to mollusk intake is mainly induced by As (Chiesa et al., 2018).

THQ values for HMs in the two species are reported in Table 4. The results show that THQ values were well below one for most of the studied metals, indicating that the exposed human population is assumed to be safe. The only exception was Cd in *N. magellanica* (THQ = 1.20), which deserves attention.

3.3. Hazard index (HI)

The HI method involves the summation of the individual THQs of the measured elements for each food type (Antoine et al., 2017; Bolt, 2019). The equation for HI is:

Table 4

Target hazard quotient (THQ) and Hazard Index (HI) values of HMs in the two sampled species (2005–2012) in the Beagle Channel (Tierra del Fuego, south Patagonia).

	Oral reference dose[a] (RfDo, μg/kg/bw/day)	Target hazard quotient (THQ)	
		M. chilensis	*N. magellanica*
Cd	1	0.30	**1.20**
Cr	3	0.07	0.13
Cu	40	0.06	0.06
Ni	20	0.02	0.003
Zn	300	0.10	0.05
Hazard Index (HI)		0.55	**1.44**

[a] Oral reference doses were obtained from US-EPA, IRIS. United States, Environmental Protection Agency, Integrated Risk Information System. Regional Screening Level (RSL) Summary Table (TR = 1E − 06, HQ = 1) May 2019 https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables (accessed September 2019). At present, there is no estimation of RfDo for Pb.

$$HI = \sum_{N=1}^{i} THQ_n$$

If the HI is > 1 there is the potential for adverse non-carcinogenic health effects. From Table 4, the HI for *M. chilensis* is considered of no health concern (< 1). On the contrary, HI for *N. magellanica* (i.e. 1.44) is of health concern. It is worth noting that Cd in *N. magellanica* accounts for 83% of the HI.

Overall, our study indicates that metals may pose a health risk only to high mussel consumers; the health risk would be particularly related to the levels of Cd and Pb in *M. chilensis* from polluted sites. This risk level is consistent with the maximum metal levels we determined for both biomonitors in the BC (see shaded boxes Table 1). These statements can also be reasonably applied to high gastropod consumers. In fact, as reported above, except for CFDA MLs, there are no suggested limits on edible gastropods (data on *N. magellanica* in Table 1 is given for indicative comparison). However, edible gastropods are an indigenous food, usually consumed only occasionally by locals or tourists in the BC, as we have confirmed during our stays in Tierra del Fuego.

The Brown Bay site (Fig. 2) is a mussel cultivation area that produces bivalves mainly for local and tourist consumption. Surprisingly, in Brown Bay, we detected the highest median Ni levels in *Mytilus* samples (3.26 μg/g ww). This result deserves attention, even if it is difficult to establish the contamination source (Conti et al., 2019b).

Nevertheless, *Nacella* was determined to be the less-safe species to consume at a high quantity at once, mainly due to its very high Cd levels. However, the estimated consumption levels of patellids are substantially lower than the estimated bivalve consumption levels (20 vs. 50 g/week).

It is worth noting that risk estimates are subject to several uncertainty factors, i.e., consumption rates can vary significantly among specific consumers in a population; in these studies, it is assumed that cooking has no effect on the elements' toxicity; and the ingested dose is considered equivalent to the absorbed dose (Primost et al., 2017; Stankovic et al., 2012; see for review Marques et al., 2011). Several critical factors regarding consumer practices and perceptions of risk around bivalve consumption have been recently reported (Crovato et al., 2019; Crovato, Pinto, Arcangeli, Mascarello, & Ravarotto, 2017). The AAs identified some relevant aspects to be considered, such as the place of purchase, the cooking and storage conditions, raw mollusk consumption and health risks, categories of at-risk consumers, and the quality control of the production chain. These aspects are also connected with the marketing of a perishable product such as fresh mussels. Some models of consumer intention to buy mussels have been proposed based on consumer information (Acebrón, Mangin, & Dopico, 2001). Another relevant factor is that the risk assessment for essential micronutrients such as Cu and Zn must take into account the two ends of the dose-response relationship, i.e., the risk of deficiency and the risk of toxicity (Goldhaber, 2003; Renwick & Walker, 2008). The Food and Nutrition Board (2001; see also Trumbo, Yates, Schlicker, & Poos, 2001) set recommended dietary allowances (RDAs), upper intake levels (ULs) and other reference values for micronutrients (i.e., Cu, Ni, Zn). Moreover, MLs can vary over time due to the discovery of new scientific evidence. That is why the consumption of bivalves with HM values close to the current MLs should always be considered with caution, i.e., the mean Pb values for *M. chilensis* we determined in the present study (1.56 μg/g ww) were close to the MLs for bivalves recommended by Mercosur, the Commission Regulation (EC), and the CFDA (1.50 μg/g ww, Tables 1–2).

4. Conclusions

In this study, we applied the MBS conceptual model to obtain reliable information on the quality for consumption of edible mollusks through a long-term survey (2005 → 2012) conducted in a remote area, i.e., the Beagle Channel (Patagonia). Six trace metals (essential and nonessential) were analyzed in two edible biomonitors (i.e mollusks) in seven hundred and twenty-nine samples collected at seven selected sites in the Beagle Channel (Patagonia). This enriched the requisite variety in order to confirm whether Patagonian mollusks are safe for consumption.

For Cd and Pb, 65% and 40% of bivalves exceeded the MLs established by Mercosur, respectively.

For Cr, Cu, Ni, Pb and Zn, mollusks were safe for consumption and did not raise concerns for public health (i.e. EDIs were lower than safety reference doses, THQ < 1 and HI < 1).

However, *N. magellanica* was the less-safe species to consume at a high quantity at once due to its high bioaccumulation levels of Cd (i.e. THQ > 1; HI > 1), occasional high consumers may be at a certain health risk.

Finally, the concept that the BC is uncontaminated should be reexamined. Furthermore, studies involving either the possible synergic effects among metals or the chemical speciation of metals are needed in order to obtain reliable information about metal intake/uptake in mollusks (Buffle, Wilkinson, & Van Leeuwen, 2009; Campbell, 1995; Muse, Carducci, Stripeikis, Tudino, & Fernández, 2006). More precisely, studies that quantify the interactions among the different metallic species and the biological receptor sites in the mollusks are needed in order to move towards a better understanding of mineral uptake/intake in seafood.

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Declaration of Competing Interest

We declare that we have no conflict of interest.

References

Acebrón, L. B., Mangin, J. P. L., & Dopico, D. C. (2001). A proposal of the buying model for fresh food products: The case of fresh mussels. *Journal of International Food & Agribusiness Marketing, 11*, 75–96.

Ashby, W. R. (1960). *An introduction to cybernetics.* London: Chapman & Hall.

Antoine, J. M., Fung, L. A. H., & Grant, C. N. (2017). Assessment of the potential health risks associated with the aluminium, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. *Toxicology Reports, 4*, 181–187.

Baki, M. A., Hossain, M. M., Akter, J., Quraishi, S. B., Shojib, M. F. H., Ullah, A. A., et al. (2018). Concentration of heavy metals in seafood (fishes, shrimp, lobster and crabs) and human health assessment in Saint Martin Island, Bangladesh. *Ecotoxicology and Environmental Safety, 159*, 153–163.

Barbosa, I. D. S., Brito, G. B., dos Santos, G. L., Santos, L. N., Teixeira, L. S., Araujo, R. G., et al. (2019). Multivariate data analysis of trace elements in bivalve molluscs: Characterization and food safety evaluation. *Food Chemistry, 273*, 64–70.

Barros Paiva, R., Neves, A., Sequeira, V., Cardoso, C., Bandarra, N., Serrano Gordo, L., et al. (2012). Risks and benefits' consumption of birdbeak dogfish Deania calcea. *British Food Journal, 114*, 826–839.

Beier, E. E., Maher, J. R., Sheu, T. J., Cory-Slechta, D. A., Berger, A. J., Zuscik, M. J., et al. (2013). Heavy metal lead exposure, osteoporotic-like phenotype in an animal model, and depression of Wnt signaling. *Environmental Health Perspectives, 121*, 97–104.

Beyer, J., Green, N. W., Brooks, S., Allan, I. J., Ruus, A., Gomes, T., et al. (2017). Blue mussels (Mytilus edulis spp.) as sentinel organisms in coastal pollution monitoring: A review. *Marine Environmental Research, 130*, 338–365.

Bigatti, G., De Vivar, M. D., Cumplido, M., Vilela, R. N., Avaro, M., Sastre, et al. (2018). Fatty acids and contaminants in edible marine gastropods from Patagonia. *Journal of the Marine Biological Association of the United Kingdom, 98*, 1355–1363.

Bolt, H. (2019). *2.7 Mixtures and Combinations of Chemicals. Toxicology and Risk Assessment: A Comprehensive Introduction* (148. 2nd. Ed.,). Wiley.

Bonsignore, M., Manta, D. S., Mirto, S., Quinci, E. M., Ape, F., Montalto, V., et al. (2018).

Bioaccumulation of heavy metals in fish, crustaceans, molluscs and echinoderms from the Tuscany coast. *Ecotoxicology and Environmental Safety, 162*, 554–562.

Buffle, J., Wilkinson, K. J., & Van Leeuwen, H. P. (2009). Chemodynamics and bioavailability in natural waters. *Environmental Science and Technology (EST), 43*, 7170–7174.

Campbell, P. G. C. (1995). Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. In A. Tessier, & D. R. Turner (Eds.). *Metal Speciation and Bioavailability in Aquatic Systems* (pp. 45–102). UK: John Wiley and Son, West Sussex.

Carvalho, D. C., Coelho, L. M., Acevedo, M. S. M., & Coelho, N. M. (2015). The oligoelements. In M. De la Guardia, & S. Garrigues (Eds.). *Handbook of mineral elements in food* (pp. 109–122). UK: John Wiley & Sons.

CFDA. China Food and Drug Administration (2017). National Food Safety Standard for Maximum Levels of Contaminants in Foods (GB 2762-2017). GAIN report number CH12085 US Department of Agriculture (22 June, 2018) < https://www.fas.usda.gov/data/china-china-releases-standard-maximum-levels-contaminants-foods > (accessed 15th September 2019).

Cheung, M. S., & Wang, W. X. (2008). Uses of subcellular metal distribution in prey to predict metal bioaccumulation and internal exposure in a predator. *Environmental Toxicology and Chemistry, 27*(5), 1160–1166.

Chiesa, L. M., Ceriani, F., Caligara, M., Di Candia, D., Malandra, R., Panseri, S., et al. (2018). Mussels and clams from the italian fish market. is there a human exposition risk to metals and arsenic? *Chemosphere, 194*, 644–649.

Codex Alimentarius (amended 2019) General Standard For Contaminants and Toxins In Food and Feed. Cxs193-1995. < http://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en/ > .

Commission Regulation (EC) (2006). No 1881/2006. (rev. 19.3.2018) Setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union L, 364*, 5–24.

Connan, O., & Tack, K. (2010). Metals in marine environment (mollusc Patella sp., fish Labrus bergylta, crustacean Cancer pagurus, beach sand) in a nuclear area, the North Cotentin (France). *Environmental Monitoring and Assessment, 165*(1–4), 67–86.

Conover, W. J. (1999). *Practical nonparametric statistics* (3rd ed.). .

Conti, M. E., & Finoia, M. G. (2010). Metals in molluscs and algae: A north–south Tyrrhenian Sea baseline. *Journal of hazardous materials, 181*(1–3), 388–392.

Conti, M. E., Canepari, S., Finoia, M. G., Mele, G., & Astolfi, M. L. (2018). Characterization of Italian multifloral honeys on the basis of their mineral content and some typical quality parameters. *Journal of Food Composition and Analysis, 74*, 102–113.

Conti, M. E., Stripeikis, J., Finoia, M. G., & Tudino, M. B. (2011). Baseline trace metals in bivalve molluscs from the Beagle Channel, Patagonia (Argentina). *Ecotoxicology, 20*(6), 1341–1353.

Conti, M. E., Stripeikis, J., Finoia, M. G., & Tudino, M. B. (2012). Baseline trace metals in gastropod mollusks from the Beagle Channel, Tierra del Fuego (Patagonia, Argentina). *Ecotoxicology, 21*(4), 1112–1125.

Conti, M. E., Tudino, M. B., Finoia, M. G., Simone, C., & Stripeikis, J. (2019a). Performance of two Patagonian molluscs as trace metal biomonitors: The overlap bioaccumulation index (OBI) as an integrative tool for the management of marine ecosystems. *Ecological Indicators, 101*, 749–758.

Conti, M. E., Tudino, M. B., Finoia, M. G., Simone, C., & Stripeikis, J. (2019b). Managing complexity of marine ecosystems: From the monitoring breakdown structure (MBS) to the baseline assessment. Trace metal concentrations in biomonitors of the Beagle Channel, Patagonia (2005–2012). *Ecological Indicators, 104*, 296–305.

Costa, M., & Klein, C. B. (2006). Toxicity and carcinogenicity of chromium compounds in humans. *Critical Reviews in Toxicology, 36*(2), 155–163.

Crovato, S., Mascarello, G., Marcolin, S., Pinto, A., & Ravarotto, L. (2019). From purchase to consumption of bivalve molluscs: A qualitative study on consumers' practices and risk perceptions. *Food Control, 96*, 410–420.

Crovato, S., Pinto, A., Arcangeli, G., Mascarello, G., & Ravarotto, L. (2017). Risky behaviours from the production to the consumption of bivalve molluscs: Involving stakeholders in the prioritization process based on consensus methods. *Food Control, 78*, 426–435.

De la Guardia, M., & Garrigues, S. (2015). *Handbook of mineral elements in food.* John Wiley & Sons.

Dorne, J. L., Kass, G. E., Bordajandi, L. R., Amzal, B., Bertelsen, U., Castoldi, A. F., et al. (2011). Human risk assessment of heavy metals: Principles and applications. *Met Ions Life Science, 8*(4), 27–60.

EFSA (2009a). European Food Safety Authority. Scientific opinion on arsenic in food. EFSA J. EFSA Journal 2009; 7(10):1351.

EFSA (2009b). European Food Safety Authority. Cadmium in food. EFSA J. 980, 1-139.

EFSA (2012). European Food Safety Authority (2012). Lead dietary exposure in the European population. EFSA Journal 2012, vol. 10, 7 (pp. 2831).

EFSA (European Food Safety Authority) (2014). Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water. EFSA Panel on Contaminants in the Food Chain (CONTAM)2, Parma, Italy. EFSA Journal 2014, vol. 12, 3 (pp. 3595).

EFSA (2015). European Food Safety Authority. Scientific opinion on the risks to public health related to the presence of nickel in food and drinking water. *EFSA Journal, 13*, 4002–4203.

FAO-WHO (2011) Evaluation of certain food additives and contaminants: seventy-third report of the Joint FAO/WHO Expert(WHO technical report series ; no. 960).

Fiorito, F., Amoroso, M. G., Lambiase, S., Serpe, F. P., Bruno, T., Scaramuzzo, A., et al. (2019). A relationship between environmental pollutants and enteric viruses in mussels (Mytilus galloprovincialis). *Environmental Research, 169*, 156–162.

Food and Nutrition Board, Institute of Medicine. (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy of Medicine.

Gil, M. N., Giarratano, E., Barros, V., Bortolus, A., Codignotto, J. O., Schenke, et al. (2019). Southern Argentina: The Patagonian Continental Shelf (783–811). In C. Sheppard (Ed.). *World Seas: An Environmental Evaluation* (pp. 783–811). NY: Academic Press.

Goldhaber, S. B. (2003). Trace element risk assessment: Essentiality vs toxicity. *Regulatory Toxicology and Pharmacology, 38*(2), 232–242.

González, N., Calderón, J., Rúbies, A., Timoner, I., Castell, V., Domingo, J. L., et al. (2019). Dietary intake of arsenic, cadmium, mercury and lead by the population of Catalonia, Spain: Analysis of the temporal trend. *Food and Chemical Toxicology, 132*, 110721.

Gupta, S. K., Singh, S. P., & Shukla, V. K. (2005). Copper, zinc, and Cu/Zn ratio in carcinoma of the gallbladder. *Journal of Surgical Oncology, 91*(3), 204–208.

Hague, T., Petroczi, A., Andrews, P. L., Barker, J., & Naughton, D. P. (2008). Determination of metal ion content of beverages and estimation of target hazard quotients: A comparative study. *Chemistry Central Journal, 2*(1), 13.

IARC (1990). International Agency for Research on Cancer, Chromium, nickel and welding. *IARC Monographs Evaluation Carcinogenic Risks Human, 49*, 49–256.

IARC (2012). International Agency for Research on Cancer, IARC Monographs, 100C vols. (IARC, Lyon, 141).

JECFA (2019).The Joint FAO/WHO Expert Committee on Food Additives, 2011 Lead evaluation. < https://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID = 3511 > .

Jović, M., & Stanković, S. (2014). Human exposure to trace metals and possible public health risks via consumption of mussels *Mytilus galloprovincialis* from the Adriatic coastal area. *Food and Chemical Toxicology, 70*, 241–251.

Larraín, M. A., Díaz, N. F., Lamas, C., Uribe, C., & Araneda, C. (2014). Traceability of mussel (*Mytilus chilensis*) in southern Chile using microsatellite molecular markers and assignment algorithms. Exploratory survey. *Food Research International, 62*, 104–110.

Liu, Q., Liao, Y., & Shou, L. (2018). Concentration and potential health risk of heavy metals in seafoods collected from Sanmen Bay and its adjacent areas, China. *Marine Pollution Bulletin, 131*, 356–364.

Liu, Q., Xu, X., Zeng, J., Shi, X., Liao, Y., Du, P., et al. (2019). Heavy metal concentrations in commercial marine organisms from Xiangshan Bay, China, and the potential health risks. *Marine Pollution Bulletin, 141*, 215–226.

Lu, G., Zhu, A., Fang, H., Dong, Y., & Wang, W. X. (2019). Establishing baseline trace metals in marine bivalves in China and worldwide: Meta-analysis and modelling approach. *Science of The Total Environment, 669*, 746–753.

Marcovecchio, J. E., De Marco, S. G., Buzzi, N. S., Botté, S. E., Labudia, A. C., La Colla, N., et al. (2015). Fish and seafood. In M. De la Guardia, & S. Garrigues (Eds.). *Handbook of mineral elements in food* (pp. 621–643). UK: John Wiley & Sons.

Marques, A., Lourenço, H. M., Nunes, M. L., Roseiro, C., Santos, C., Barranco, A., et al. (2011). New tools to assess toxicity, bioaccessibility and uptake of chemical contaminants in meat and seafood. *Food Research International, 44*(2), 510–522.

Mercosur (2011). Reglamento técnico MERCOSUR sobre límites máximos de contaminantes inorgánicos en alimentos. RES. N° 12/11, Grupo Mercado Comun. Secretaría de Políticas, Regulación e Institutos y Secretaría de Agricultura, Ganadería y Pesca. Código Alimentario Argentino. < www.puntofocal.gov.ar/doc/r_gmc_12-11.pdf > (accessed 21st June 2019).

Mok, J. S., Kwon, J. Y., Son, K. T., Choi, W. S., Kang, S. R., Ha, N. Y., et al. (2014). Contents and risk assessment of heavy metals in marine invertebrates from Korean coastal fish markets. *Journal of Food Protection, 77*(6), 1022–1030.

Muse, J. O., Carducci, C. N., Stripeikis, J. D., Tudino, M. B., & Fernández, F. M. (2006). A Link Between Lead and Cadmium Kinetic Speciation in Seawater and Accumulation by the Green Alga Ulva Lactuca. *Environmental Pollution, 141*(1), 126–130.

Naughton, D. P., & Petróczi, A. (2008). Heavy metal ions in wines: Meta-analysis of target hazard quotients reveal health risks. *Chemistry Central Journal, 2*(1), 22.

Needleman, H. (2004). Lead poisoning. *Annual Review of Medicine, 209*, 209–222.

Nieto Vilela, R. A., Cumplido, M., Giorgis, Y. G., Gil, M. N., & Bigatti, G. (2019). Reproduction and nutritional values of the edible limpet *Nacella magellanica* (Gastropoda: Patellogastropoda). *Scientia Marina, 83*(3).

Olmedo, P., Pla, A., Hernández, A. F., Barbier, F., Ayouni, L., & Gil, F. (2013). Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environment International, 59*, 63–72.

Özden, Ö., & Erkan, N. (2011). A preliminary study of amino acid and mineral profiles of important and estimable 21 seafood species. *British Food Journal, 113*(4), 457–469.

Pastorelli, A. A., Baldini, M., Stacchini, P., Baldini, G., Morelli, S., Sagratella, E., et al. (2012). Human exposure to lead, cadmium and mercury through fish and seafood product consumption in Italy: A pilot evaluation. *Food Additives & Contaminants: Part A, 29*(12), 1913–1921.

Patrick, L. (2006a). Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. *Alternative Medicine Review, 11*(1), 2–22.

Patrick, L. (2006b). Lead toxicity part II: The role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. *Alternative Medicine Review, 11*(2), 114–127.

Pérez, A. A., Fajardo, M. A., Farías, S. S., Strobl, A. M., Camarda, S., Garrido, B., et al. (2017). Metals and metalloids in mussels from the Argentine Patagonia. *International Journal of Environment and Health, 8*(4), 290–301.

Petroczi, A., & Naughton, D. (2009). Mercury, cadmium and lead contamination in seafood: A comparative study to evaluate the usefulness of Target Hazard Quotients. *Food and Chemical Toxicology, 47*(2), 298–302.

Peycheva, K., Panayotova, V., & Stancheva, M. (2016). Assessment of human health risk for copper, arsenic, zinc, nickel, and mercury in marine fish species collected from Bulgarian black sea coast. *International Journal of Fisheries and Aquatic Studies, 4*(5), 41–46.

Phillips, D. J. (1995). The chemistries and environmental fates of trace metals and organochlorines in aquatic ecosystems. *Marine Pollution Bulletin, 31*(4–12), 193–200.

Pino, A., Alimonti, A., Conti, M. E., & Bocca, B. (2010). Iridium, platinum and rhodium baseline concentration in lichens from Tierra del Fuego (South Patagonia, Argentina). *Journal of Environmental Monitoring, 12*(10), 1857–1863.

Primost, M. A., Gil, M. N., & Bigatti, G. (2017). High bioaccumulation of cadmium and other metals in Patagonian edible gastropods. *Marine Biology Research, 13*(7), 774–781.

Ragi, A. S., Leena, P. P., Cheriyan, E., & Nair, S. M. (2017). Heavy metal concentrations in some gastropods and bivalves collected from the fishing zone of South India. *Marine Pollution Bulletin, 118*(1–2), 452–458.

Reilly, C. (1991). *Metal contamination of food* (2nd. ed). London: Elsevier Applied Science Publishers.

Renwick, A. G., & Walker, R. (2008). Risk assessment of micronutrients. *Toxicology Letters, 180*(2), 123–130.

Ruiz-Fernández, A. C., Wu, R. S., Lau, T. C., Pérez-Bernal, L. H., Sánchez-Cabeza, J. A., & Chiu, J. M. (2018). A comparative study on metal contamination in Estero de Urias lagoon, Gulf of California, using oysters, mussels and artificial mussels: Implications on pollution monitoring and public health risk. *Environmental Pollution, 243*, 197–205.

Sfriso, A. A., Chiesa, S., Sfriso, A., Buosi, A., Gobbo, L., Gnolo, A. B., et al. (2018). Spatial distribution, bioaccumulation profiles and risk for consumption of edible bivalves: A comparison among razor clam, Manila clam and cockles in the Venice Lagoon. *Science of The Total Environment, 643*, 579–591.

Simon, H. A. (1962). The architecture of complexity. *Proceedings of the American Philosophical Society, 106*(6), 467–482.

Simone, C., Barile, S. (Eds.) (2015) “Environment, Health and Business Management: Linking efficiency and effectiveness to viable sustainability”, International Journal of Environment and Health, vol. 7(4). (Special issue).

Simone, C., Barile, S. (Eds.) (2016) “Environment, Health and Business Management: Linking efficiency and effectiveness to viable sustainability”, International Journal of Environment and Health, speciali issues vol. 8(1). (Special issue).

Stankovic, S., Jovic, M., Stankovic, A. R., & Katsikas, L. (2012). *Heavy metals in seafood mussels. Risks for human health. In Environmental chemistry for a sustainable world.* Dordrecht: Springer311–373.

Szefer, P., & Nriagu, J. O. (2007). *Mineral components in foods.* CRC Press.

Tellez-Plaza, M., Jones, M. R., Dominguez-Lucas, A., Guallar, E., & Navas-Acien, A. (2013). Cadmium exposure and clinical cardiovascular disease: A systematic review. *Current Atherosclerosis Reports, 15*(10), 356.

Trumbo, P., Yates, A. A., Schlicker, S., & Poos, M. (2001). Dietary reference intakes: Vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *Journal of the Academy of Nutrition and Dietetics, 101*(3), 294.

US EPA (2019) Human Health Risk Assessment: Risk-Based Concentration Table. Regional Screening Levels (RSLs) Summary Table (TR = 1E-06, HQ = 1) May, 2019. < https://www.epa.gov/risk/regional-screening-levels-rsls-generic-tables > .

US FDA (2019) U.S. Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition. 4th Edition August 2019. < www.FDA.gov/Seafood > .

USEPA (2007). US Environmental Protection Agency. Framework for metals risk assessment. US Environmental Protection Agency, Office of the Science Advisor: Washington, DC. EPA 120/R-07/001.

Velez, C., Figueira, E., Soares, A., & Freitas, R. (2015). Spatial distribution and bioaccumulation patterns in three clam populations from a low contaminated ecosystem. *Estuarine, Coastal and Shelf Science, 155*, 114–125.

Ventura, M., Cardoso, C., Bandarra, N. M., Delgado, I., Coelho, I., Gueifão, S., et al. (2018). Bromine, arsenic, cadmium, and lead in several key food groups: An assessment of relative risk. *International Journal of Environmental Analytical Chemistry, 98*(15), 1398–1412.

WHO (World Health Organization), 1996. Trace elements in human nutrition and health (A report of a re-evaluation of the role of trace elements in human health and nutrition). Geneva. < http://whqlibdoc.who.int/publications/1996/9241561734_eng.pdf > .

Wilson, C. (2014). Semi-structured interviews. *Interview Techniques for UX Practitioners, 1*, 23–41.

Yusà, V., & Pardo, O. (2015). Human risk assessment and regulatory framework for minerals in food. In M. De la Guardia, & S. Garrigues (Eds.). *Handbook of mineral elements in food* (pp. 69–108). UK: John Wiley & Sons.

Yüzereroğlu, T. A., Gök, G., Coğun, H. Y., Firat, Ö., Aslanyavrusu, S., Maruldalı, O., et al. (2010). Heavy metals in Patella caerulea (Mollusca, Gastropoda) in polluted and non-polluted areas from the Iskenderun Gulf (Mediterranean Turkey). *Environmental monitoring and assessment, 167*(1–4), 257–264.

Zand, N., Christides, T., & Loughrill, E. (2015). Dietary intake of minerals. In M. De la Guardia, & S. Garrigues (Eds.). *Handbook of mineral elements in food* (pp. 23–39). UK: John Wiley & Sons.

Zaza, S., de Balogh, K., Palmery, M., Pastorelli, A. A., & Stacchini, P. (2015). Human exposure in Italy to lead, cadmium and mercury through fish and seafood product consumption from Eastern Central Atlantic Fishing Area. *Journal of Food Composition and Analysis, 40*, 148–153.

Zhang, Y. F., Chen, S. Y., Qu, M. J., Adeleye, A. O., & Di, Y. N. (2017). Utilization of isolated marine mussel cells as an in vitro model to assess xenobiotics induced genotoxicity. *Toxicology in Vitro, 44*, 219–229.