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To cite this article: Mónica A. Primost, Mónica N. Gil & Gregorio Bigatti (2017): High bioaccumulation of cadmium and other metals in Patagonian edible gastropods, Marine Biology Research, DOI: [10.1080/17451000.2017.1296163](https://doi.org/10.1080/17451000.2017.1296163)

To link to this article: <http://dx.doi.org/10.1080/17451000.2017.1296163>



Published online: 08 Jun 2017.



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High bioaccumulation of cadmium and other metals in Patagonian edible gastropods

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ABSTRACT

High concentrations of metals are accumulated by edible marine resources, affecting human health. Marine gastropods have been commercially captured and consumed in Argentina for decades without official regulations or studies to guarantee their safety. In this study, metals (cadmium, aluminium, iron, zinc, copper and lead) were analysed for the first time in the edible marine gastropods *Buccinanops globulosus*, *Adelomelon ancilla* and *Trophon geversianus* and their surrounding sediments. Taking into account the maximum levels recommended by national and international regulations for food safety, we recommend consuming only the foot of these gastropod species. We recommend not consuming these gastropods from harbour areas due to concentrations of lead in sediments and tissues. Though the target hazard quotient (THQ) of cadmium was not high for the local community, it could be higher for other populations who consume molluscs more frequently, indicating a potential health risk of chronic exposure. Monitoring of these and other contaminants should be performed in order to ensure food safety of these marine resources.

ARTICLE HISTORY

Received 4 November 2016
Accepted 9 February 2017

RESPONSIBLE EDITOR

Sharon Hook

KEYWORDS

Cadmium; human risk;
marine pollution;
Neogastropoda; Patagonia

Introduction

Trace elements naturally occur in the sea, while environmental levels may be increased by natural processes as well as anthropogenic activities. Marine organisms incorporate metals from their surroundings, depending on abiotic factors as well as on the characteristics of the species, the exposure period and the concentration and chemical speciation of the elements (Langston et al. 1998). As a result, biota can accumulate metals in high concentrations and human health can be at risk because of consumption of contaminated seafood.

Marine molluscs, in particular gastropods, have widely been used as bioindicators, since several negative effects are evident as a consequence of exposure to endocrine disrupters in polluted environments (Meador et al. 1995). Metals have been reported in tissues of marine gastropods such as *Nucella lapillus* (Linnaeus, 1758), *Reisha clavigera* (Küster, 1860), *Tritia reticulata* (Linnaeus, 1758) and other species (Bryan et al. 1987; Blackmore & Morton 2002; Wang & Ke 2002; Gust et al. 2011). In general, the digestive gland has been the target of study due to its involvement in metabolic processes and its role in detoxification

(Regoli & Orlando 1994; Marigomez et al. 2002; Wang & Ke 2002). Since most marine gastropods are edible, many authors have also assessed their potential risk to human consumption due to their ability to accumulate toxic metals (Conti et al. 2012a; Copat et al. 2013; Bat et al. 2016; Lahbib et al. 2016).

On the marine coast of Patagonia Argentina, there have been many studies of metal accumulation in abiotic and biotic components (Gil et al. 2006; Conti et al. 2011; Giarratano et al. 2013). However, metal content in marine gastropods has only been reported for the limpet *Nacella magellanica* (Gmelin, 1791) in the Beagle Channel, at the southernmost tip of Argentina (Conti et al. 2012b).

This study was conducted on the west coast of Nuevo Gulf, in Northern Atlantic Patagonia. The most important economic activities in the region are fisheries, aluminium production and tourism based on local fauna such as the whale *Eubalaena australis* (Desmoulins, 1822). An important maritime activity has also been developed within Nuevo Gulf. Various species of edible gastropods belonging to Nassariidae, Muricidae and Volutidae inhabit this coastal zone. We studied three species: *Adelomelon ancilla* (Lightfoot, 1786),

which feeds on live bivalves and other gastropod species, and inhabits mixed sediments (Zabala et al. 2013a); *Buccinanops globulosus* (Kiener, 1832) (= *Buccinanops deformis* (King, 1832) *sensu* Scarabino et al. 2006), which feeds on carrion (Bökenhans 2014) and lives buried in muddy sediments (Scarabino 1977); and *Trophon geversianus* (Pallas, 1774), which inhabits the bivalve complex *Brachidontes rodriguezii* (d'Orbigny, 1842)/*Perumytilus purpuratus* (Lamarck, 1819) and feeds on them. These gastropods are locally consumed and commercialized albeit without official regulations or safety quality controls (Narvarte 2006; Bigatti & Ciocco 2008; Bigatti et al. 2015). Between 2000 and 2004, the annual catch of *B. globulosus* in San Matías Gulf was up to 9 tons with an estimated price per kilogram in 2015 of US\$7.7.

Metals such as zinc and copper are nutritional elements, although they can be toxic in high concentrations. Iron is widely recognized as essential to marine organisms (Butler 1998) and aluminium has no known biological function (Exley & Mold 2015). Lead and cadmium are considered to be toxic even at low concentrations (WHO 2006). In this paper, we determined for the first time the metal contents (cadmium (Cd), aluminium (Al), iron (Fe), Zinc (Zn), copper (Cu) and lead (Pb)) in the edible gastropods *B. globulosus*, *A. ancilla* and *T. geversianus* and explored their potential risk for human health through dietary intake. The main substrate where each species lives and feeds (sediments and small mussels, *B. rodriguezii*/*P. purpuratus*) was also analysed.

Sediment toxicity to biota was assessed by comparison of measured concentrations with national and international guidelines.

Material and methods

Study area and sampling

Samples were collected in Nuevo Gulf, at four sites with different levels of anthropogenic activity: Storni harbour (SH), Luis Piedra Buena harbour (LPBH) and the beaches of Punta Cuevas (PC) and Cerro Avanzado (CA) (Figure 1). The level of anthropogenic activity at these sites has been assessed previously. There are two harbours characterized by the presence of commercial, fishing and recreational vessels (~700 vessels per year) (APPM 2016). Moderate levels of trace metals, polycyclic aromatic hydrocarbons (PAHs) and tributyltin (TBT) were previously recorded in sediments and bivalve molluscs (Gil et al. 1999; Massara Paletto et al. 2008; Bigatti et al. 2009). The PC site is a recreational area; low trace metals and TBT pollution were reported in sediments and marine organisms (Bigatti et al. 2009; Di Salvatore et al. 2013; Giarratano et al. 2013). In contrast, CA is a site with scarce vessel traffic and where diving activities are occasionally performed. It has been considered as a control site in pollution studies related to hydrocarbons, tributyltin and booster biocides (Bigatti et al. 2009; Del Brio et al. 2016). Three gastropod species were collected in a unique sampling event. Approximately 30 specimens

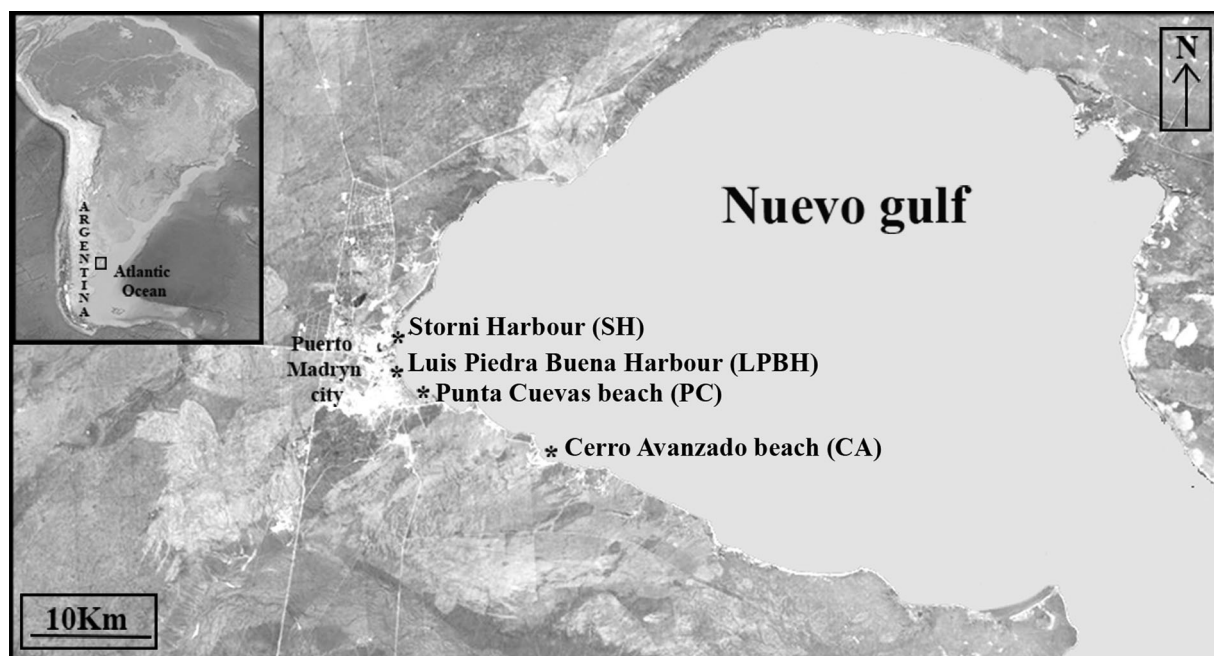


Figure 1. Sampling sites in Nuevo Gulf.

of *Trophon geversianus* and *Buccinanops globulosus* (LPBH, PC and CA sites) as well as five specimens of *Adelomelon ancilla* (SH site) were collected by scuba diving in July 2011. The low number of *A. ancilla* individuals collected was due to their scarcity and slow growth. They were still included because this species is normally consumed locally. Sediment samples (up to 10 cm depth) were collected using plastic cores, at the sites inhabited by *A. ancilla* and *B. globulosus*, which live buried in this substrate most of the time. Small-sized mussels, *B. rodriguezii* and *Perumytilus purpuratus*, were collected from rocky shores where *T. geversianus* lives and feeds on these tiny mussels (LPBH, PC and CA, N = 20 at each site).

Sample processing

Bulk sediments were dried at 60°C. Granulometry was performed by passing the sample through 2 mm and 63 µm meshes. For further analysis, particles larger than 2 mm were discarded. Organic matter was determined by loss on ignition at 450°C for 4 h. For analysis of metals, 1 g of dry sediment was treated with Aqua Regia according to Agemian & Chau (1976). This extraction method has been considered useful for evaluating the potential of aquatic sediments for biological effects (CCME 2001). Gastropods were measured (0.1 mm precision digital caliper) and weighed (0.001 g digital scale) and the digestive gland-gonad complex and foot were carefully separated for subsequent analysis. In the case of the bivalves that constitute the diet of *Trophon geversianus*, analyses were performed on total soft tissues.

Each tissue type (foot and gland-gonad complex of gastropods, and whole soft tissue of bivalves) was grouped by site and then oven dried at 60°C. Percentage moisture was calculated as the loss of weight after drying. Dried material was homogenized in an agate mortar; 1 g of homogenate was placed in a crucible and calcinated in a muffle furnace (Fisher 186). Temperature was slowly increased from room temperature to 400°C for 6 h. The next day, 2 ml of concentrated HNO₃ was added and evaporated to dryness on a sand bath (Thermoline Type 2200) at 80°C. This procedure was repeated until white ashes were obtained. Ashes were resuspended with 4 ml of acid solution (HNO₃ 3% v/v and HCl 6% v/v) and made up to 10 ml.

All analyses were performed in triplicate and each set was accompanied by two blanks consisting of the reagents treated in the same way as the samples. The extracts were stored at 4°C, until metal determination in an IL-457 atomic absorption spectrophotometer

(AAS). An air-acetylene flame was used, except for Al which was measured using a nitrous oxide-acetylene flame. Unless otherwise indicated, results are reported as µg g⁻¹ of dry wet (for sediments) and wet weight (for biota).

The total metal content (TMC) in both gastropod tissues was estimated as the weighted average of the individual contents (Foot + Digestive Gland-Gonad complex), as follows:

$$\text{TMC}(\mu\text{g}\cdot\text{g}^{-1}) = [M]_{(F)} \times \frac{F \text{ weight}}{F + \text{DG} - G \text{ weight}} + [M]_{(\text{DG}-G)} \times \frac{\text{DG} - G \text{ weight}}{F + \text{DG} - G \text{ weight}}$$

where [M]_(F) and [M]_(DG-G) are the mean metal concentrations in the foot and in the complex Digestive Gland-Gonads, respectively. Results were reported as µg g⁻¹ of wet weight.

Reagents of analytical grade (Merck) were used for the blanks and for the calibration curves. Quality assurance of sediment and organism results was done through analysis of the standard reference marine sediment PACS-2 and Oyster Tissue 1566b, respectively. For sediment samples, detection limits were 0.25 (Cd), 5 (Pb), 1.25 (Cu), 0.25 (Zn), 2.5 (Fe %) and 10 (Al %) µg g⁻¹. Recovery percentage in reference material 'Marine Sediment PACS-2' was between 81 and 102%, and the variation coefficient was between 1.3 and 16.3%. For tissue samples, detection limits were 0.03 (Cd), 0.5 (Pb), 0.3 (Cu), 0.45 (Zn), 1.0 (Fe) and 6.0 (Al) µg g⁻¹. Recovery percentage in reference material 'Oyster Tissue 1566b' was between 76 and 105%, and the variation coefficient was between 1.2 and 9.7%.

Health risk assessment

Depending on the size and species, gastropods can be consumed whole or only the muscular foot. In this study, we assessed the potential human health risk of the studied metals due to consumption of all the gastropod tissues as well as only the foot. For this purpose, we compared Total Metal Content (TMC) of Cd and Pb with maximum levels (ML) recommended by national and international regulations (SENASA 2000; MERCOSUR/GMC 2011; WHO 2006). No limits have been suggested in these regulations for the other metals. We also estimated the required intake of gastropods to reach the provisional tolerable intake of most recently (2011–2013) evaluated metals by the JECFA: Cd (PTMI: 25 µg/kg bw/month) and Al (PTWI: 2 mg/kg bw/week). Lead was not included, since previously established PTWI was withdrawn by

the Committee in 2011. The Target Hazard Quotient (THQ) provides an indication of the risk level due to exposure to contaminants (USEPA 1989). In order to assess the non-carcinogenic health risk from consuming gastropods for the local population, target hazard quotients (THQ) were calculated as follows:

$$\text{THQ} = \frac{\text{EF} \cdot \text{ED} \cdot \text{C} \cdot \text{FIR}}{\text{RFD} \cdot \text{WAB} \cdot \text{TA}} \cdot 0.001$$

where EF is the frequency of exposure (days/year), ED is the exposure duration (years), FIR is the rate of food intake (g/person/day), C is the metal concentration in seafood ($\mu\text{g g}^{-1}$), RFD is the oral reference dose provided by the EPA's Integrated Risk Information System (IRIS) online database (https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0141_summary.pdf), WAB is the body weight (kg) and TA is the average time of exposure to non-carcinogenic compounds (365 days/year \times ED). Estimations were only performed for Cd (RFD = 1.0×10^{-3} mg kg^{-1} /day), since RFD for Al and Pb are not considered in the IRIS. For Argentinean consumers the EF was fixed at 48 days/year; ED 70 years; FIR 2.32 g/person/day; and WAB 72 kg (Walpole et al. 2012).

For the purposes of this paper, in accordance with the EPA guidelines, it was assumed that cooking has no effect on the toxicity of elements and the ingested dose is equal to the absorbed contaminant dose.

Results and discussion

Metals in sediments

Sediments showed oxidizing or partially oxidizing characteristics, with fine-sandy particles. The highest organic matter content was found at PC (Table I), a site that is characterized by high macroalgal biomass, resulting from drift and accumulation during north winds (Gil et al. 2014). The highest metal concentrations were measured in LPBH (Table I), in accordance with the highest amount of sediment particles below 63 μm and the influence of the harbour activity. The general pattern of metal concentration in sediment samples was $\text{Fe} > \text{Al} > \text{Zn} > \text{Cu} > \text{Pb} > \text{Cd}$. Concentrations of Fe and Al found in this study were higher

than those previously reported by Giarratano et al. (2013) in Nuevo Gulf, probably due to the different granulometry of the analysed samples. Fe and Al are geochemically abundant elements in the earth's crust and their oxyhydroxide phases may be carriers of other metals in the marine environment (Wen et al. 2008). The maximum values measured for Zn, Cu and Pb were similar to those found by other authors in other Patagonian gulfs (Harvey & Gil 1988; Giarratano et al. 2013; Marinho et al. 2013). The levels in sediments were below international guidelines for biota protection (USEPA 1977; CCME 2001). Cd was undetectable in all sediment samples, as previously reported in most coastal systems from Patagonia (Argentina).

Metals in the diet of Trophon geversianus (Brachidontes rodriguezii–Perumytilus purpuratus)

All the metals were detected in the tiny mussels *Brachidontes rodriguezii–Perumytilus purpuratus*, with the exception of Pb (Table II). Concentrations followed the pattern: $\text{Fe} \sim \text{Al} > \text{Zn} > \text{Cu} > \text{Cd} > \text{Pb}$ and, despite the proximity of the aluminium factory, they were in most cases below the values previously reported for the bivalve *Aulacomya atra* (Molina, 1782) living in the same areas (Gil et al. 1988; Giarratano et al. 2013). This result could be related to the exclusion of gills in the analysed tissues (Bebiano & Serafim 1998).

Metals in gastropods

Metal concentrations were detected in foot and digestive gland-gonad complex of the three gastropod species (Figure 2). Cd was found in the gastropods *Adelomelon ancilla* and *B. globulosus*, which live most of the time buried in fine sediments, despite not being detected in the corresponding sediment matrix. Unlike other transition metals, sediment diagenesis is an important factor controlling Cd bioavailability. This metal migrates into pore water in the top oxidized sediment layer while the inverse occurs in the reduced deeper layers (Rosenthal et al. 1995), which is the most likely explanation of why this metal is not

Table I. Metal levels (mean \pm SD) ($\mu\text{g g}^{-1}$ dw), grain size (%) and organic matter (OM) (%) in marine sediments from sampling sites.

Site	Al	Fe	Zn	Cu	Cd	Pb	Grain size			OM
							C	M	F	
SH	5633 \pm 866.4	12,179 \pm 478.7	19.36 \pm 0.88	2.50 \pm 0.0003	ND	5.00 \pm 0.007	20.76	43.56	35.67	2.5
LPBH	12,958 \pm 44.6	13,581 \pm 290.7	32.99 \pm 3.3	6.10 \pm 0.2	ND	7.47 \pm 0.005	0.01	19.82	80.16	1.74
PC	8664 \pm 184.6	12,175 \pm 1068.3	18.92 \pm 0.7	2.74 \pm 0.3	ND	ND	0.12	70.21	29.65	10.48
CA	10,541 \pm 507.9	10,492 \pm 84.5	16.84 \pm 0.9	2.99 \pm 0.001	ND	ND	0.02	70.58	29.39	1.09

Note: Abbreviations: C: coarse (>2 mm), F: fine (<63 μm), M: medium (63 μm < grain < 2 mm), ND: non-detectable concentration; CA: Cerro Avanzado beach, LPBH: Luis Piedra Buena harbour, PC: Punta Cuevas beach, SH: Storni harbour.

Table II. Metal levels ($\mu\text{g g}^{-1}\text{dw}$) in gastropods and bivalves from the studied sites.

Species	Site	Al		Fe		Zn		Cu		Cd		Pb	
Bivalves:													
<i>Brachidontes rodriguezii</i>	LPBH	130.15		193.53		74.66		9.19		1.13		ND	
<i>Perumytilus purpuratus</i>	PC	282.83		396.12		43.79		16.97		1.83		ND	
	CA	300.37		94.61		19.75		2.26		0.52		ND	
		F	DG-G	F	DG-G	F	DG-G	F	DG-G	F	DG-G	F	DG-G
Gastropods:													
<i>Adelomelon ancilla</i>	SH	ND	ND	13.93	723.63	27.01	781.65	9.81	586.31	0.06	8.02	ND	1.81
	LPBH	23.2	13.04	290.0	526.71	70.29	1091.15	24.95	59.73	0.36	53.12	1.63	2.61
<i>Buccinanops globulosus</i>	PC	103.32	13.31	244.98	476.82	59.11	959.42	16.49	45.21	0.20	38.88	1.02	1.95
	CA	22.69	17.21	82.85	621.69	77.29	787.09	17.26	55.81	0.29	190.24	1.02	1.98
	LPBH	21.39	ND	184.31	351.57	199.43	583.56	21.49	68.93	0.76	58.82	1.17	10.37
<i>Trophon geversianus</i>	PC	71.89	ND	181.97	400.80	183.09	504.52	29.77	75.41	1.23	104.24	ND	3.51
	CA	54.01	15.55	131.69	298.01	136.00	296.06	19.48	37.38	0.79	64.78	ND	1.94

Abbreviations: F: foot, DG-G: Digestive Gland-Gonad complex, ND: non-detectable concentrations; CA: Cerro Avanzado beach, LPBH: Luis Piedra Buena harbour, PC: Punta Cuevas beach, SH: Storni harbour.

always well correlated in sediments and organisms (Apeti et al. 2009). Therefore, the most probable sources of Cd in *A. ancilla* and *B. globulosus* would be the surrounding pore water and food; further studies should include the analysis of Cd in these two substrates, the surrounding pore water and food of the gastropods, in order to advance our understanding of the uptake routes.

The digestive gland-gonad complex was the main storage tissue for all measured metals, except for Al in *B. globulosus* and *T. geversianus* (Table II). The digestive gland-gonad complex was proposed in previous works as a target for metal detoxification in many invertebrate species (Rainbow 2007), while

metallothionein induction is reported as an effective detoxification mechanism in other marine invertebrates (Cheung et al. 2002; Khati et al. 2012).

The levels of TMC found in this study were in general higher than concentrations found in bivalves (except for Fe and Al) from the same region (Giarratano et al. 2013). In comparison to other species of gastropods, TCM levels in the harbour area were similar or slightly higher than those found in *Nacella magellanica* from the Beagle Chanel, Argentina and lower (except for Cd) than those found in different species of gastropods around the world such as *Patella caerulea* Linnaeus, 1758, *Littorina littorea* (Linnaeus, 1758), *Buccinum undatum* Linnaeus, 1758, *Haliotis tuberculata* Linnaeus,

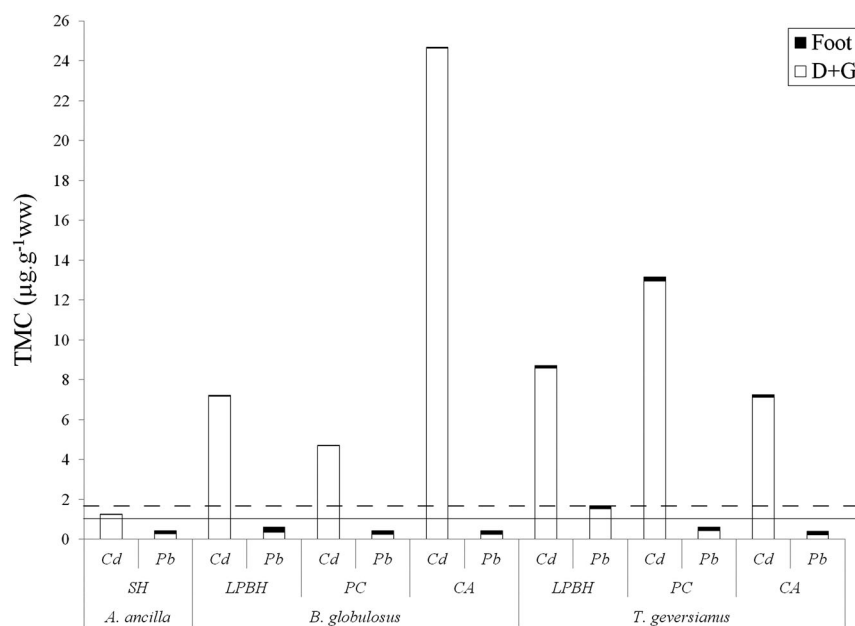


Figure 2. Total metal content ($\mu\text{g.g}^{-1}\text{ww}$) in gastropods (foot and digestive gland + gonad complex). Recommended values for Cd (continuous line) and Pb (dashed line) in samples of mollusc for human consumption (SENASA 2000; MERCOSUR/GMC 2011; WHO 2006) are indicated. Abbreviations: CA: Cerro Avanzado beach, LPBH: Luis Piedra Buena harbour, PC: Punta Cuevas beach, SH: Storni harbour.

1758 and *Bolinus brandaris* (Linnaeus, 1758), among others (Yüzereroğlu et al. 2010; Noël et al. 2011; Bille et al. 2015; Lahbib et al. 2016).

Health risk assessment

Taking into account the recommendations of SENASA (2000), MERCOSUR/GMC (2011) and WHO (2006) for samples of molluscs destined for human consumption, all samples exceeded the recommended maximum levels of Cd (Figure 2), and only *T. geversianus* in LPBH exceeded the levels of Pb.

Regarding the provisional tolerable intake, a monthly ingestion of 1.2 kg of *Adelomelon ancilla*, 0.065 kg of *B. globulosus* or 0.12 kg of *Trophon geversianus* entire gastropod tissues would be enough to reach the PTMI for Cd (25 µg/kg bw/month). However, if only the foot tissues were consumed, ingested amounts to reach that limit would increase towards 83, 15 and 4.4 kg for *A. ancilla*, *B. globulosus* and *T. geversianus* respectively. In the case of Al, and considering that the foot presented the highest concentrations, 7 kg of this tissue of *B. globulosus* or 10.3 kg from *T. geversianus* could be consumed in a week to reach the established PTWI (2 mg/kg bw/week).

According to these estimations, *B. globulosus* would be the less safe species to consume, mainly due to Cd levels. However, the highest THQ obtained for this metal was 0.1, much lower than 1, which would mean that the daily exposure at the measured level would be unlikely to cause any health risks. However, if exported to countries where the consumption of marine gastropods is higher, the THQ values should be re-calculated, as these increase with the food intake ratio.

On the other hand, *A. ancilla* seems to be the safest species for human consumption. However, Zabala et al. (2013b) have recommended not to use it as a fishing resource in Nuevo Gulf, because of its slow growth, low densities and low fertility, and this must be taken into account.

Conclusions

This study suggests that according to local consumption habits, dietary intake of the studied gastropods is probably safe. However, it must be kept in mind that Cd and Pb concentrations exceeded maximum levels (mainly in the harbour area) and that risk may increase if the food intake ratio is enhanced. In order to protect public health, monitoring of toxic metals and other pollutants in fishing resources for human

consumption should be sustained over time by local authorities.

Acknowledgements

Special thanks to Carmen Marinho from Centro para el Estudio de los Sistemas Marinos (CESIMAR) for her assistance with sample processing. Ricardo Vera, Fabián Quiroga, Néstor Ortiz and Julio Rúa helped with sampling. This is publication No. 82 of the Laboratorio de Reproducción y Biología Integrativa de Invertebrados Marinos (LARBIM).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was partially supported by the projects PICT 1232, PICT 2929, PICT 2709, PICT 1491, PICT 1476, PIP 067 and the PADI foundation.

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