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Bioaccumulation and biomagnification of potentially toxic elements in the octopus *Octopus hubbsorum* from the Gulf of California

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ABSTRACT

The concentrations of 21 potentially toxic elements (PTEs) were determined in the tissues of *Octopus hubbsorum* from three locations along the Gulf of California coast: two near Santa Rosalia (SR), a site with historical metal contamination, and one in La Paz Bay, a reference site. Concentrations of Cd, Co, Cr, Mn, Ni, Pb, and Zn in octopus from the two SR sites were higher than those from the reference site, reflecting the higher sediment concentrations at the mining-impacted locations. The highest bioaccumulation and biomagnification of elements was found in digestive gland and branchial hearts, while the lowest was observed in the mantle, where the mean concentration of PTEs did not exceed international standards for human consumption of octopus. This study found elevated PTEs in octopus from sites with high metal contamination, and presents the first data on these elements in octopus from the Gulf of California.

1. Introduction

Octopus is a high-demand fishery for human consumption throughout the world (FAO, 2016). However, similar to other cephalopods, they have the ability to bioaccumulate high concentrations of potentially toxic elements (PTEs) in their tissues, posing a risk to their consumers (e.g. Napoleão et al., 2005; Rjeibi et al., 2014). For this reason, understanding PTEs in these organisms has become a topic of growing international interest (e.g. Raimundo et al., 2010a; Semedo et al., 2012; Grayson and Sekadende, 2014; Karim et al., 2016).

In marine ecosystems, octopuses play a key role in the transfer of contaminants through food webs (Bustamante et al., 1998a). They feed upon crustaceans and molluscs (FAO, 2016) that accumulate high concentrations of metals and other elements (Jakimska et al., 2011), and are known to accumulate potentially toxic elements (PTEs) such as Cd and As (Seixas et al., 2005a). Octopuses are also part of the diet of many predators including fishes, marine mammals and seabirds, so they act as vectors in the transfer of PTEs to higher trophic levels (Bustamante et al., 1998a, b).

The bioaccumulation of PTEs in octopus occurs differentially in the various organs and tissues, with the digestive gland and branchial hearts as the main storage and detoxification sites (Nessim and Riad, 2003; Raimundo and Vale, 2008; Raimundo et al., 2010a). Other tissues such as the mantle and arms - the only parts consumed by humans - generally have lower concentrations (e.g. Napoleão et al., 2005; Raimundo and Vale, 2008). However, there are reports that the mantle and arms of octopus exceed the maximum PTE content in international standards for human consumption, posing a risk for human health (Rossi et al., 1993; Grayson and Sekadende, 2014).

In Mexico, the octopus fishery on the Pacific coast is mainly based on the capture of a single species: *Octopus hubbsorum* (López-Uriarte et al., 2005). Within the Gulf of California, the most important location for this fishery is the Santa Rosalia mining port (López-Uriarte et al., 2005), a site known to have potentially toxic levels of metals in coastal marine sediments (Shumilin et al., 2013).

Despite their potential to affect human and ecosystem health, to date there are no studies evaluating PTE concentrations in octopus from Santa Rosalia, nor in any octopus species inhabiting coastal areas of the American continent. The objectives of this research were to determine: (1) the bioaccumulation of PTEs in four tissues of *Octopus hubbsorum*: mantle, branchial hearts, digestive gland, and gills; and (2) the biomagnification of these elements, if any, by analyzing one of its potential

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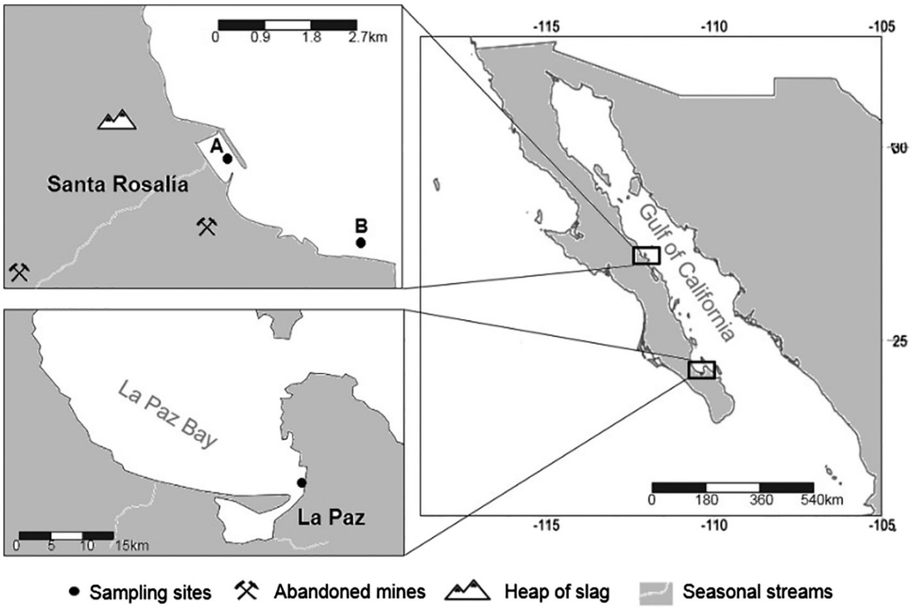

Fig. 1. Sampling sites of *Octopus hubbsorum* on the Gulf of California: two sites near Santa Rosalía (A and B) with historical metal contamination in marine sediments, and one in La Paz Bay, as a reference site.

prey, the clam *Megapitaria squalida*, collected from the Santa Rosalia mining port. Results from the mining-impacted sites were compared to the same species from La Paz Bay, BCS, Mexico, a reference site.

2. Materials and methods

2.1. Study area

Santa Rosalia is a fishing port located in the central part of the Gulf of California's western coast, Mexico (Fig. 1). The local climate is arid, with a mean temperature of 23.4 °C, and scarce rainfall, with annual mean precipitation of 117.2 mm (Volke-Sepúlveda et al., 2003). It is a location with a long history of mining activity related to copper extraction from “El Boleo” field (1885–1985), so that the local coastal sediments are highly polluted by metals, mainly Mn, Cu, Zn, Pb, Co, U and Cd (Shumilin et al., 2013). The concentrations of these elements are so high that they are potentially toxic for marine biota (Shumilin et al., 2013). Specifically, Cu concentrations exceeded the effect range medium (ERM) by up to 17-fold, which translates into a potential negative biological effect for 50% of marine organisms (Shumilin et al., 2011). The local mussels *Modiolus capax* (Gutiérrez-Galindo et al., 1999; Muñoz-Barbosa and Huerta-Díaz, 2013) and *Mytilus edulis* (Cadena-Cárdenas et al., 2009) have Cu concentrations up to 11 times higher than those from other areas of the Baja California peninsula; both bivalve species are part of the diet of *O. hubbsorum* (López-Uriarte et al., 2010).

2.2. Sampling

Cd, Co, Cu, Mn, Pb, and Zn levels in Santa Rosalia marine sediments show a marked decreasing gradient toward the port's most remote areas (Shumilin et al., 2011, 2013). Accordingly, sampling was conducted in two locations: at (A) the area closest to the port dock (Hot spot zone), where the highest sediment concentrations of these elements have been recorded, and at (B) an area located 2 km to the south, where lower sediment concentrations have been found (Fig. 1). In addition, reference samples were collected from El Caimancito, La Paz Bay. This site is 400 km south of Santa Rosalia and sediment metal concentrations here are related mainly to the regional geology and deemed typical of pristine environments with no anthropogenic impact; the effects of the closest mining area (the mining company Roca Fosfórica Mexicana “Rofomex”) are localized (Rodríguez-Castañeda et al., 2006) (Fig. 1).

Twenty three *O. hubbsorum* specimens were collected from the following locations: Santa Rosalia A (n = 8), Santa Rosalia B (n = 7) and La Paz Bay (n = 8). Specimens were caught by artisanal fishermen in October 2015 and June 2016. To avoid any potential bias from individuals of extreme size or weight, all octopuses were within a similar range of size and weight. Specimens were transported in sea water with ice to reduce their metabolic rate and minimize the mobilization of PTEs between organs and tissues (Martin and Flegal, 1975). Immediately after being killed, the sex, total weight (TW), total length (TL), and dorsal mantle length (DML) were determined. The mantle (mantle and arms are the main tissues consumed by humans but mantle is more frequently used in similar studies), digestive gland (main storage tissue), branchial hearts (main detoxification tissue) and gills (tissue with major interaction with water and exposed to the waterborne contamination) of each specimen was removed. Each tissue was carefully washed with deionized water, weighed (± 0.1 g), frozen (− 20 °C), freeze-dried (− 30 °C and 100 m Torr), powdered and homogenized in agate mortars for analysis. To minimize the risk of contamination, organisms and samples were handled only with acrylic and plastic materials previously washed with deionized water and Milli-Q(R) metal-free water with 5% nitric acid. Analyses were performed in the Canadian Rivers Institute at the University of New Brunswick, NB, Saint John, Canada.

2.3. Determination of potentially toxic elements

Using between 0.001 and 0.02 g (dry weight) of each sample, total Hg (THg) was determined by thermal decomposition, amalgamation and thermal absorption, with a Milestone DMA-80 direct mercury analyzer. Quality assurance was assessed every 10 samples using a blank ($< 0.00250\ \mathrm{mg\ kg^{-1}}$ limit of detection), a duplicate (relative percent difference $< 20\%$), a mussel tissue certified reference material SRM 2976 and an internal reference material (recovery percentages between 80 and 120%). The limit of detection (LOD) was determined using 3 × the SD of the blanks run over the project plus the average of the blanks (see Table 1).

For the determination of Ag, Al, As, Cd, Co, Cr, Cu, Fe, La, Mg, Mn, Ni, Pb, Rb, Se, Sr, Tl, U, V and Zn, approximately 0.5 g (dry weight) of each sample was digested with 10 mL of concentrated nitric acid (HNO_3) in a CEM MARS5 microwave digester for approximately 1 h. Then, each sample was diluted in 40 mL Milli-Q(R) metal-free water, and was analyzed by inductively coupled plasma optical emission

Table 1
Detection limits, concentrations of potentially toxic elements in the certified reference material SRM 2976 (mean ± SD) and percentage recovery according the concentrations obtained.

Element	Detection limit ($mg\ kg^{-1}$, dry weight)	SRM 2976 ($mg\ kg^{-1}$, dry weight)	Recovery (%)
Ag	0.22	0.011 ± 0.005	98
Al	0.31	134 ± 34	111
As	1.60	13.3 ± 1.8	103
Cd	0.13	0.82 ± 0.16	107
Co	0.15	0.61 ± 0.02	89
Cr	0.12	0.50 ± 0.16	108
Cu	0.19	4.02 ± 0.33	96
Fe	0.68	171.0 ± 4.9	97
Hg	0.01	61.0 ± 3.6	79
La	1.00	n. c.	–
Mg	4.25	0.53 ± 0.05	92
Mn	0.10	33.0 ± 2.0	108
Ni	0.13	0.93 ± 0.12	98
Pb	0.77	1.19 ± 0.18	104
Rb	0.86	4.14 ± 0.09	93
Se	1.08	1.80 ± 0.15	105
Sr	0.004	93.0 ± 2.0	84
Tl	0.55	n. c.	–
U	6.37	n. c.	–
V	0.15	n. c.	–
Zn	1.02	137 ± 13	97

n. c. - no certified.

spectrometry (ICP-OES), using 1.5 mL of 29% lithium nitrate as an ionization buffer and 100 μL of yttrium (Y, 1000 mg/L) as the internal standard. The accuracy and precision were determined from reading the point of calibration (1.0 and 20.0 ppm) before and after each batch of samples (recovery percentage between 80 and 120%). A batch of samples consisted of method blank (≤ detection limit), 12 samples, a duplicate (relative percent difference < 20%) and mussel tissue as a certified reference material SRM 2976 (recovery percentage between 79 and 111%). The detection limit (DL) was calculated as 5 times the instrument detection limit (IDL); the IDL was determined by running 20 repeats of a blank and then adding 3 times the SD to the average (see Table 1).

The concentrations of each element including Hg are reported in $mg\ kg^{-1}$ dry weight.

2.4. Analyses of data

Considering that the digestive gland is the main site of PTE bioaccumulation in cephalopods (Miramand and Guary, 1980), the extent to which accumulation in this tissue was greater than mantle muscle was determined. To this end, we used the classification proposed by Miramand and Bentley (1992), where the ratio of the concentration of each element in the digestive gland relative to the mantle (DG:M) indicates whether the element is highly concentrated (ratio > 50), moderately concentrated (50 > ratio > 10) or slightly concentrated (ratio < 10). In addition, since branchial hearts are also major PTE bioaccumulation sites (Mangold et al., 1989), the same classification was applied to determine the extent to which PTE concentration in this organ was greater than that of the mantle (BH:M).

To evaluate biomagnification in *O. hubbsorum*, we used PTE concentrations in the clam *Megapitaria squalida*, a common prey in the diet of this octopus (personal observation) as well as in the diet of other local octopuses such as *Octopus bimaculatus* (Armendáriz-Villegas et al., 2014). Data were obtained from sampling conducted in 2013 at three similar locations: Santa Rosalia port (an area with a high metal concentrations) (n = 4), 7 km north of Santa Rosalia (an area with lower metal concentrations) (n = 4), and La Paz Bay (n = 2). The analyses were done on whole body clams, using the same analytical procedures described above. Biomagnification was assessed by calculating the biomagnification factor (BMF) for each site, as follows: PTE concentration in *O. hubbsorum* (C_2), relative to the mean concentration in the prey *M. squalida* (C_1), expressed as $BMF = C_2/C_1$ (Newman, 2014).

Table 2
Size and weight (mean ± SD, range) of *Octopus hubbsorum* from three locations in the Gulf of California.

	Santa Rosalía A	Santa Rosalía B	La Paz Bay	ANOVA
Dorsal mantle length (cm)	8.6 ± 1.4 (7–11)	8.6 ± 1.3 (7–11)	9.0 ± 1.5 (6.5–11)	$F_{2,23} = 0.25$, p = 0.78
Total length (cm)	35.5 ± 4.4 (28–39)	34.9 ± 2.8 (32–40)	37.1 ± 6.3 (28–44)	$F_{2,23} = 0.46$, p = 0.64
Total weight (g)	375 ± 167 (228–673)	438 ± 120 (270–577)	478 ± 157 (286–710)	$F_{2,23} = 0.95$, p = 0.40

Any potential differences in PTE concentrations between sexes, organs and localities were examined using one-way analyses of variance (ANOVA) and Kruskal-Wallis nonparametric tests, according to compliance with the assumptions of normality and homogeneity of variances, checked through the Kolmogorov-Smirnov and Bartlett tests, respectively. To determine the source of variation, a posteriori Tukey and Bonferroni tests were used after ANOVA and Kruskal-Wallis tests, respectively. All statistical analyses were performed in the software STATISTICA for Windows (version 12.0, Stat Soft 2013), with a 95% confidence level ($\alpha = 0.05$).

3. Results

The size and weight of the octopuses examined ranged from 7 to 11 cm mantle length, 28 to 44 cm total length, and 228 to 710 g total weight. La Paz Bay specimens displayed the highest values of all measurements; however, no significant differences were found in size and weight between the three sampling localities (ANOVA, $p > 0.05$) (Table 2).

3.1. Concentration of potentially toxic elements in tissues

The metal concentrations that were determined in octopus from these sites included nine elements in the mantle (Al, As, Cu, Fe, Hg, Mg, Mn, Sr and Zn); 17 in the digestive gland (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Pb, Sr, V and Zn); 16 in the branchial hearts (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Se, Sr, U, V and Zn); and 14 in the gills (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Se, Sr, and Zn). It was not possible to determine the concentration of all 21 elements in each tissue as some were below the detection limit. Ag and Pb were detected only in the digestive gland, and U was found only in the branchial hearts. La, Rb and Tl were below the detection limits in all tissues (< 1.00, < 0.86 and < 0.55 $mg\ kg^{-1}$, respectively). The mean concentrations and ranges for each element by site and tissue are shown in Table 3.

3.2. Effect of sex on the concentration of potentially toxic elements

Of the 23 octopus specimens examined, 39% were females and 61% were males, with a female: male sex ratio of 1:1.5; the ratio by site was 1:3 (Santa Rosalia A), 1:1.3 (Santa Rosalia B) and 1:1 (La Paz Bay). PTE concentrations in the tissues showed no significant differences between males and females in each site ($p > 0.05$), suggesting that sex did not affect PTE accumulation in organisms within the size/weight ranges examined. Thus, the data from both sexes were pooled for subsequent analyses.

3.3. Differences in PTE concentrations between tissues

A comparison of PTE concentrations between octopus tissues across

Table 3
Concentration of potentially toxic elements (mg kg^{-1}, dry weight) in mantle, digestive gland, branchial hearts and gills of *Octopus hubbsorum* and other octopus species.

Tissue	Species	Locality	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Hg	La	Reference
Muscle (mantle) *Mantle and arms	*Octopus hubbsorum*	Santa Rosalía	< 0.22	3.7 (1.1–7.5)	37 (14–55)	< 0.13	< 0.15	< 0.12	25 (6–37)	10 (1 – 20)	0.07 (0.02–0.25)	< 1.00	Present study
**Arms		La Paz Bay	< 0.22	5.6 (0.5–11)	65 (25–93)	< 0.13	< 0.15	< 0.12	20 (14–29)	9 (5–15)	0.13 (0.07–0.24)	< 1.00	Present study
	Octopus vulgaris	Portugal							3.9–72				Raimundo et al., 2004
		Portugal*				0.04–3.3			5.5–72	6.5–81			Raimundo et al., 2005
		Portugal*			13–87				30 ± 19	24 ± 15			Napoleão et al., 2005
		Affric				23			36 (17–106)	32 (11–84)			Soldevilla, 1987
		Monaco				0.08 ± 0.04			26 ± 2	30 ± 5			Miramand and Guary, 1980
		Portugal				0.25–4.4			8.3–62	17–43			Raimundo and Vale, 2008
		Portugal									0.27–0.48		Seixas et al., 2005b
		Portugal**			40–133	0.4–19			7.9–81	15–49	0.15–0.43		Seixas et al., 2005a
		Egypt				1.3–1.8	1.7–1.9	5.8–5.9	5.2–12.6	10.5–15.6			Nessim and Riad, 2003
		Tunisia	< 0.03			0.15–0.5			20–40		0.1–0.4		Rjeibi et al., 2014
		Morocco				0.24–0.58	1.2–2.7		133–171	40.6–229			Karim et al., 2016
	Graneledone sp.	Indian Ocean				0.37			15				Bustamante et al., 1998b
	Benthoctopus thielei	Indian Ocean				0.21			3				Bustamante et al., 1998b
	Eledone cirrhosa	France	0.05			0.24 ± 0.01	< 0.1	0.7 ± 0.4	17 ± 1	25 ± 10			Miramand and Bentley, 1992
	Eledone cirrhosa	Italy									0.5–9.0		Rossi et al., 1993
Digestive gland	*Octopus hubbsorum*	Santa Rosalía	0.6 (0.3–1.3)	8 (1–37)	43 (21–142)	76 (21–252)	50 (11–109)	0.24 (0.1–0.5)	3296 (726–5880)	314 (162–644)	0.29 (0.07–1.06)	< 1.00	Present study
		La Paz Bay	2.7 (1.6–3.1)	20 (3–44)	46 (30–72)	53 (24–99)	6 (3 – 10)	0.49 (0.2–0.7)	2104 (537–4800)	454 (250–878)	0.22 (0.15–0.27)	< 1.00	Present study
	Octopus vulgaris	Portugal							137–3142				Raimundo et al., 2004
		Portugal				20–269			137–1465	292–1202			Raimundo et al., 2005
		Portugal			16–81				1768 ± 1010	790 ± 343			Napoleão et al., 2005
		Affric				34–218			82 (8.6–286)	456			Soldevilla, 1987
		Monaco				50 ± 10			2500 ± 700	700 ± 130			Miramand and Guary, 1980
		Portugal				140–555			4200				Raimundo et al., 2010a
		Portugal				94–185			28–762	142–384			Raimundo and Vale, 2008
		Portugal									0.58–3.43		Seixas et al., 2005b
		Egypt				9.1–36.1	1.9–6.1	6.8–10.9	390–715	138–179			Nessim and Riad, 2003
		Tunisia	10–28			30–90			800–1000		0.5–3		Rjeibi et al., 2014
		Morocco				1.35–1.95	0.2–7.7		152–187	104–344			Karim et al., 2016
	Graneledone sp.	Indian Ocean				369			1092				Bustamante et al., 1998b
	Benthoctopus thielei	Indian Ocean				215			42				Bustamante et al., 1998b
	Eledone cirrhosa	France	1.7–4.6			21.4–26.5	1.9–2.1	0.5–0.9	436–483	255–288			Miramand and Bentley, 1992
Branchial hearts	*Octopus hubbsorum*	Santa Rosalía	< 0.22	36 (15–50)	26 (12–59)	43 (10 – 102)	321 (104–597)	4 (1–7)	103 (26–166)	1028 (156–5340)	0.06 (0.03–0.1)	< 1.00	Present study
		La Paz Bay	< 0.22	36 (14–62)	44 (28–62)	12 (6–29)	25 (12–46)	2 (1–5)	89 (66–120)	618 (300–1580)	0.09 (0.06–0.1)	< 1.00	Present study
	Octopus vulgaris	Portugal			7.2–43				188 ± 68	577 ± 323			Napoleão et al., 2005
		Monaco				0.3 ± 0.1			500 ± 40	650 ± 150			Miramand and Guary, 1980
		Portugal				0.03–88			92–274	95–1270			Raimundo and Vale, 2008
		Portugal									0.27–0.52		Seixas et al., 2005b
		Egypt				2.4–3.7	2.4–5.4	6.1–9.5	57–121	40.8–69.3			Nessim and Riad, 2003
	Graneledone sp.	Indian Ocean				24.6			465				Bustamante et al., 1998b
	Benthoctopus thielei	Indian Ocean				31.5			306				Bustamante et al., 1998b
	Eledone cirrhosa	France	0.17			0.60	0.36	0.4	335 ± 14	21 ± 2			Miramand and Bentley, 1992
Gills	*Octopus hubbsorum*	Santa Rosalía	< 0.22	4 (0.08–8)	33 (17–73)	0.7 (0.2–1.3)	2.2 (0.6–4.8)	0.28 (0.2–0.3)	136 (84–187)	91 (16–529)	0.05 (0.02–0.11)	< 1.00	Present study
		La Paz Bay	< 0.22	7 (1–17)	47 (36–63)	0.4 (0.3–0.6)	0.2 (0.21–0.23)	0.24 (0.21–0.28)	130 (73–174)	44 (30–65)	0.08 (0.04–0.14)	< 1.00	Present study
	Octopus vulgaris	Portugal			7.6–85				113 ± 47	40 ± 28			Napoleão et al., 2005

(*continued on next page*)

Table 3 (*continued*)

Tissue	Species	Locality	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Hg	La	Reference
		Affric				17			177(92–253)	49 (19–87)			Soldevilla, 1987
		Monaco				0.05 ± 0.01			300 ± 40	40 ± 20			Miramand and Guary, 1980
		Portugal				0.19–0.90							Raimundo et al., 2010a
		Portugal				0.16–22			66–128	10–252			Raimundo and Vale, 2008
		Portugal									0.28–0.42		Seixas et al., 2005b
		Egypt				1.2–1.9	0.8–1.9	2.7–4.8	23–38	10.8–15.8			Nessim and Riad, 2003
		Morocco				0.37–0.58	0.26–3.18		371–644	208–407			Karim et al., 2016
	Graneledone sp.	Indian Ocean				22.6			530				Bustamante et al., 1998b
	Benthoctopus thielei	Indian Ocean				49.1			168				Bustamante et al., 1998b
	Eledone cirrhosa	France	0.11			0.56	< 0.1	0.7	268 ± 8	9 ± 3			Miramand and Bentley, 1992

Tissue	Species	Locality	Mg	Mn	Ni	Pb	Rb	Se	Sr	Tl	U	V	Zn	Reference
Muscle (mantle)	*Octopus hubbsorum*	Santa Rosalía	2741	1.3	< 0.13	< 0.77	< 0.86	< 1.08	13	< 0.55	< 6.37	< 0.15	58	Present study
*Mantle and arms			(923–3770)	(0.3–2.6)					(2–19)				(19–74)	
**Arms		La Paz Bay	3164	0.9	< 0.13	< 0.77	< 0.86	< 1.08	14	< 0.55	< 6.37	< 0.15	64	Present study
Muscle (mantle)			(2420–4910)	(0.7–1.1)					(9–28)				(58–73)	
	Octopus vulgaris	Portugal				0.06–2.3							37–119	Raimundo et al., 2004
		Portugal*											53–121	Raimundo et al., 2005
		Portugal*		1.8 ± 0.7	1.7 ± 1.1			0.9–2.4					76 ± 22	Napoleão et al., 2005
		Affric		2.2									70 (41–186)	Soldevilla, 1987
		Monaco		3 ± 2								0.3 ± 0.2	70 ± 30	Miramand and Guary, 1980
		Portugal				0.3–1.6							47–64	Raimundo and Vale, 2008
		Portugal**		1.5–2.39		2.9–4.0		1.1–4.2					58–142	Seixas et al., 2005a
		Egypt		1.1–1.4	8.6–11.6	1.8–7.4							13.2–19.9	Nessim and Riad, 2003
		Tunisia				< 3.2							50–120	Rjeibi et al., 2014
		Morocco				0.6–8.9							280–298	Karim et al., 2016
	Octopus cyanea	Tanzania*				2.5–8.5								Mshana and Sekadende, 2014
	Graneledone sp.	Indian Ocean											113	Bustamante et al., 1998b
	Benthoctopus thielei	Indian Ocean											138	Bustamante et al., 1998b
	Eledone cirrhosa	France		1.0 ± 0.3	0.4 ± 0.1	0.11 ± 0.05						< 0.5	105 ± 4	Miramand and Bentley, 1992
Digestive gland	*Octopus hubbsorum*	Santa Rosalía	1893	17	1.3	6.2	< 5.0	8	21	< 5.0	< 6.37	1.9	877	Present study
			(1300–2630)	(3–37)	(0.5–2.8)	(1–14)		(4–19)	(16–31)			(0.5–4.3)	(226–1500)	
		La Paz Bay	2014	6	2.4	2.7	< 5.0	8	32	< 5.0	< 6.37	2.0	802	Present study
			(1300–2620)	(3–7)	(1–4.5)	(0.7–5.9)		(5–10)	(16–48)			(0.5–2.7)	(468–1190)	
	Octopus vulgaris	Portugal				0.04–44							198–14,718	Raimundo et al., 2004
		Portugal											198–14,721	Raimundo et al., 2005
		Portugal		5.6 ± 2.4	5.4 ± 4.2	6.9 ± 3.2		4.8–16.2				3.8 ± 2	1463 ± 726	Napoleão et al., 2005
		Portugal					2.5–8.9					0.3–14.4		Seixas and Pierce, 2005
		Affric		4.2									258 (113–437)	Soldevilla, 1987
		Monaco		7 ± 0.5								4.5 ± 1	1450 ± 400	Miramand and Guary, 1980
		Portugal											12,752–48,051	Raimundo et al., 2010a
		Portugal				0.7–3.5							81–667	Raimundo and Vale, 2008
		Egypt		0.6–4.0	8.8–20.5	5.6–16.4							109–142	Nessim and Riad, 2003
		Tunisia				5–6							500–650	Rjeibi et al., 2014
		Morocco				6.7–63							17.9–80.3	Karim et al., 2016
	Octopus cyanea	Tanzania				3.6–12								Mshana and Sekadende, 2014
	Graneledone sp.	Indian Ocean											102	Bustamante et al., 1998b
	Benthoctopus thielei	Indian Ocean											416	Bustamante et al., 1998b
	Eledone cirrhosa	France		2.8–5.4	2.3–2.6	1–1.3						2.8–4.1	567–711	Miramand and Bentley, 1992
Branchial hearts	*Octopus hubbsorum*	Santa Rosalía	2635	52	263	< 0.77	< 5.0	6	61	< 0.55	17	41	130	Present study
			(828–3330)	(19–115)	(73–364)			(1–14)	(19–93)		(6–29)	(13–55)	(56–330)	
		La Paz Bay	2844	11	186	< 0.77	< 5.0	6	50	< 0.55	18	33	158	Present study
			(2330–3240)	(5–40)	(107–328)			(4–11)	(41–84)		(9–26)	(16–50)	(119–280)	

(*continued on next page*)

Table 3 (*continued*)

Tissue	Species	Locality	Mg	Mn	Ni	Pb	Rb	Se	Sr	Tl	U	V	Zn	Reference
	Octopus vulgaris	Portugal		7.8 ± 4.8	251 ± 122	8.1 ± 5.0		5.2–13.3				36 ± 17	81 ± 23	Napoleão et al., 2005
		Portugal					7–12					17–78		Seixas and Pierce, 2005
		Portugal				0.39–3.9							68–386	Raimundo and Vale, 2008
		Monaco		13 ± 3								25 ± 2	65 ± 15	Miramand and Guary, 1980
		Egypt		0.9–2.8	34.2–74.4	8.27–9.41							13–58	Nessim and Riad, 2003
	Graneledone sp.	Indian Ocean											126	Bustamante et al., 1998b
	Benthoctopus thielei	Indian Ocean											172	Bustamante et al., 1998b
	Eledone cirrhosa	France		4.5 ± 0.2	6.1 ± 0.2	0.39 ± 0.04						6.0 ± 1.0	130 ± 1	Miramand and Bentley, 1992
Gills	*Octopus hubbsorum*	Santa Rosalía	2489 (1620–2850)	7 (5–9)	0.8 (0.3–2.8)	< 0.77	< 5.0	4 (3–6)	14 (9–18)	< 0.5	< 6.37	< 0.15	87 (81–93)	Present study
		La Paz Bay	2530 (2060–3050)	6 (4–6)	0.3 (0.2–0.5)	< 0.77	< 5.0	4 (3–6)	16 (11 – 23)	< 0.5	< 6.37	< 0.15	80 (74–96)	Present study
	Octopus vulgaris	Portugal		6.3 ± 2.3	1.5 ± 0.5								72 ± 17	Napoleão et al., 2005
		Affric		7.6									68 (44–94)	Soldevilla, 1987
		Monaco		10 ± 0.5								0.5 ± 0.2	120 ± 20	Miramand and Guary, 1980
		Portugal											726–912	Raimundo et al., 2010a
		Portugal				0.2–1.1							76–122	Raimundo and Vale, 2008
		Egypt		0.1–1.1	5.5–8.4	2.1–4.4							10–20	Nessim and Riad, 2003
		Morocco				1.57–76							56–461	Karim et al., 2016
	Graneledone sp.	Indian Ocean											98	Bustamante et al., 1998b
	Benthoctopus thielei	Indian Ocean											147	Bustamante et al., 1998b
	Eledone cirrhosa	France		4.3 ± 0.1	0.7 ± 0.1	0.62 ± 0.01						< 0.5	133 ± 1	Miramand and Bentley, 1992

all sites revealed general patterns in their accumulation (Table 3). The digestive gland had the highest concentrations of Cd (21–252 mg kg^{-1}), Cu (537–5880 mg kg^{-1}), Hg (0.07–1.06 mg kg^{-1}), Pb (0.7–14 mg kg^{-1}), and Zn (226–1500 mg kg^{-1}) ($p < 0.05$), with mean values being three (Hg) to 122 (Cu) times higher than those in all other tissues. Branchial hearts showed the highest levels of Al (14–62 mg kg^{-1}), Co (12–597 mg kg^{-1}), Cr (1–7 mg kg^{-1}), Mn (5–115 mg kg^{-1}), Ni (73–364 mg kg^{-1}), Sr (19–93 mg kg^{-1}) and V (13–55 mg kg^{-1}) ($p < 0.05$), with mean concentrations being two (Sr) to 358 (Co) times higher than in the other tissues. The mantle was the tissue with the lowest concentrations of all the elements ($p < 0.05$), with mean values from one time (As, Hg, Mg) up to 122 times (Cu) lower than those in the other tissues. Element concentrations in gills were similar to (Al, As, Co, Cr, Cu, Fe, Hg, Mg, Mn, Ni, Sr, Zn) or higher (Cd, Se) than those in the mantle (one to seven-fold), and always lower than those in the digestive gland and branchial hearts.

In the digestive gland of *O. hubbsorum* from all localities, Cd and Cu were highly concentrated (DG:M ratio = 524 and 129, respectively), Zn was moderately concentrated (DG:M ratio = 16), and Pb and Hg were slightly concentrated (DG:M ratio = 6 and 3, respectively), as per the classification of Miramand and Bentley (1992). With regards to the branchial hearts, Co, Ni and V were highly concentrated (BH:M ratio = 535, 1712 and 257, respectively), Al, Cr and Mn were moderately concentrated (BH:M ratio = 11, 32 and 41, respectively), and Sr was slightly concentrated (BH:M ratio = 6).

3.4. *Differences in PTE concentrations between localities*

There were no significant differences in PTEs within octopus tissues from the two Santa Rosalia localities ($p > 0.05$). However, in one or both localities (A and/or B) some organs had concentrations (Cd, Co, Cr, Mn, Ni, Pb and Zn) that were significantly higher than those in the same tissues from La Paz Bay. These differences were found in the digestive gland (Co, Mn, and Pb), branchial hearts (Cd, Co, Cr, Mn and Ni) and gills (Cd, Cr, Mn and Zn) ($p < 0.05$), but never in the mantle ($p > 0.05$) (Figs. 2 and 3). In contrast, specimens from La Paz Bay showed significantly higher concentrations of Ag, As, Cr, Hg, Ni and Sr when compared to one or both Santa Rosalia sites. More specifically, differences were found in at least one of the organs: mantle (As and Hg), digestive gland (Ag, Cr, Ni and Sr), branchial hearts (As and Hg) and gills (As) ($p < 0.05$) (Figs. 2 and 3).

With few exceptions, the ratios of most PTEs between digestive gland-mantle and branchial hearts-mantle were similar among sites, according to the classification of Miramand and Bentley (1992). However, in Santa Rosalia Co was more concentrated in the digestive gland and Mn in the digestive gland and branchial hearts; in La Paz Bay, Ag and Al were more concentrated in the digestive gland.

3.5. *Estimation of biomagnification*

According to the BMFs, all *O. hubbsorum* organs showed some degree of biomagnification, mainly in Santa Rosalia specimens (Table 4). Some elements (Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn) in the digestive gland and branchial hearts of *O. hubbsorum* were 1 to 300 times higher than those of whole *M. squalida*. The mantle also had BMFs > 1 but with a smaller number of elements (Cu, Mn and Zn); concentrations of these PTEs were 1 to 3 times higher than those recorded in whole *M. squalida*. In addition, biomagnification of some elements was also evident when concentrations in *O. hubbsorum* were compared with literature reports of PTEs for other molluscs from the same localities (Table 5).

3.6. *Allowable PTE limits in octopus for human consumption*

In accordance with Mexican (NOM-129-SSA1-1995 and NOM-242-SSA1-2009) and international standards (Codex Alimentarius

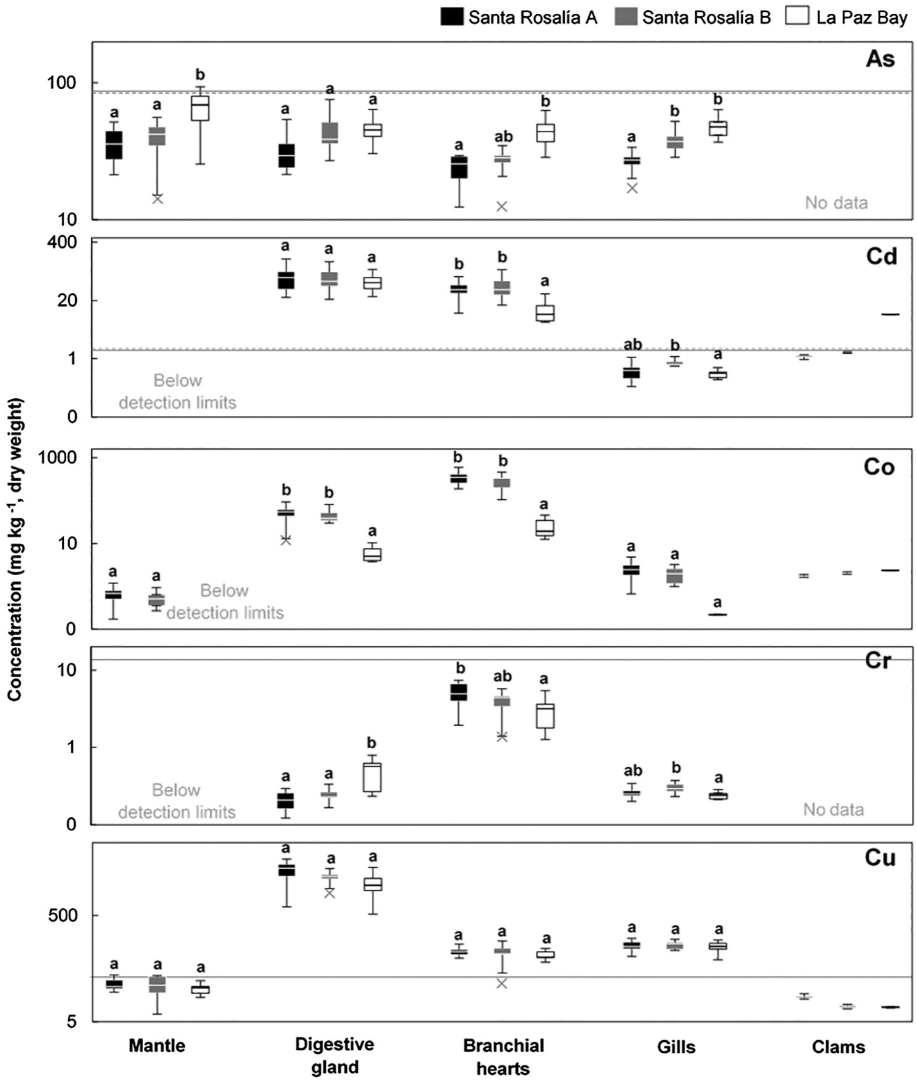

Fig. 2. Median, 25 and 75% percentiles, minimum, maximum and outliers (x) of the concentrations (note log scale) of As, Cd, Co, Cr and Cu (mg kg^{-1}, dry weight) in mantle, digestive gland, branchial hearts and gills of *Octopus hubbsorum* and whole body of the clam *Megapitaria squalida* (clams) from three locations: Santa Rosalía A and B, and La Paz Bay. Letters (a, ab, b) indicate the groups with significant differences ($p < 0.05$) between locations for each tissue. Horizontal lines indicate the standards for human consumption, according to international standards CODEX, EC and FDA (solid line) and to the Mexican government (dashed line).

Commission, European Community and US-Food and Drug Administration) (Table 6), average As, Cd, Cr, Cu, Hg, Ni and Pb concentrations in *O. hubbsorum* mantle from Santa Rosalia A and B, and La Paz Bay were below the maximum allowable limits for human consumption (Figs. 2 and 3).

4. Discussion

4.1. PTE concentrations in O. hubbsorum

PTE concentrations in tissues of *O. hubbsorum* from these sites in the Gulf of California were within the ranges reported for other octopus species in the world (Table 3). The only exceptions were Co, Fe and Mn in the digestive gland and branchial hearts, mainly in Santa Rosalia specimens, which exceeded by up to 100-fold (Co) the maximum values reported for *O. vulgaris* (e.g. Soldevilla, 1987; Nessim and Riad, 2003). In contrast, Co, Cr and Ni in the mantle of *O. hubbsorum* showed concentrations up to 48 times (Cr) lower than the minimum levels reported for *O. vulgaris* (Nessim and Riad, 2003; Napoleão et al., 2005).

In the current study, the elements with the highest concentrations in *O. hubbsorum* were Mg > Cu > Fe > Zn. This finding is consistent with essential elements that are abundant in marine organisms (Jakimska et al., 2011), including octopuses like *O. vulgaris* (e.g. Raimundo et al., 2004; Napoleão et al., 2005; Seixas et al., 2005a; Raimundo et al., 2010a). For Mg, as well as for Al, La, Sr and Tl, no comparisons could be made because this is the first report on their concentrations in octopus.

With respect to sex, the bioaccumulation of PTEs in *O. hubbsorum* was similar in tissues of males and females, which is consistent with the findings reported for *E. cirrhosa* and *O. vulgaris* (e.g. Raimundo et al., 2004; Seixas et al., 2005b; Raimundo and Vale, 2008; Rjeibi et al., 2014).

4.2. Differences in PTE bioaccumulation between tissues

In *O. hubbsorum*, the higher concentrations of Cu > Zn > Cd > Pb > Hg in the digestive gland versus other tissues were consistent with those reported for *O. vulgaris* (e.g. Raimundo et al., 2004; Seixas et al., 2005b; Karim et al., 2016), and supports the observation that this organ is the primary site for final storage and excretion of PTEs in bivalves, gastropods (Marigómez et al., 2002), and cephalopods (Miramand and Guary, 1980), since food is considered as the main source of PTEs in this organisms (Bustamante et al., 2000).

According to the classification of Miramand and Bentley (1992), Cd and Cu are the elements with the highest concentrations in the digestive gland of *O. hubbsorum*, followed by Zn, Hg and Pb. This rank in concentrations is in accordance with the concentrations reported for the digestive gland of *O. vulgaris* (Raimundo et al., 2004; Raimundo and Vale, 2008), and is related to the function of chelating agents in this organ, especially cytosolic proteins (Penicaud et al., 2017). In

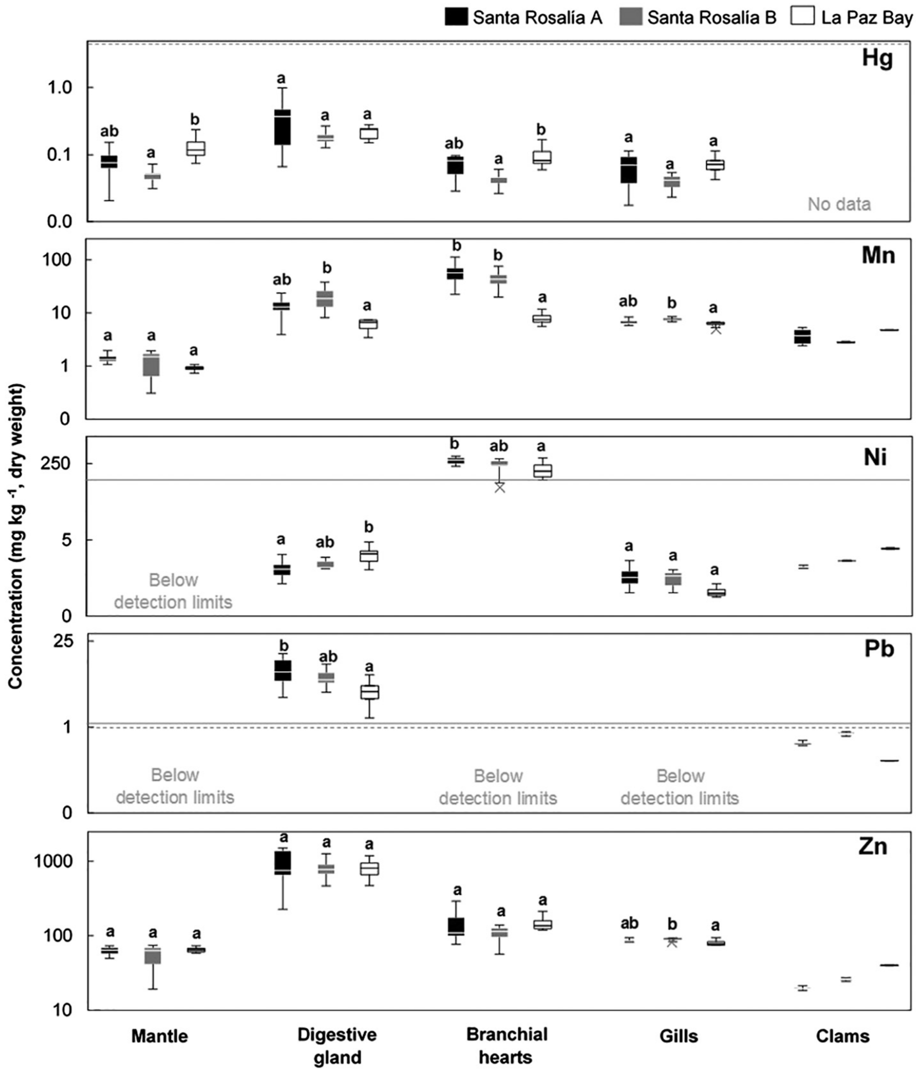

Fig. 3. Median, 25 and 75% percentiles, minimum, maximum and outliers (x) of the concentrations (note log scale) of Hg, Mn, Ni, Pb and Zn (mg kg^{-1}, dry weight) in mantle, digestive gland, branchial hearts and gills of *Octopus hubbsorum* and whole body of the clam *Megapitaria squalida* (clams) from three locations: Santa Rosalía A and B, and La Paz Bay. Letters (a, ab, b) indicate the groups with significant differences ($p < 0.05$) between locations for each tissue. Horizontal lines indicate the standards for human consumption, according to international standards CODEX, EC and FDA (solid line) and to the Mexican government (dashed line).

Table 4
Biomagnification factors of potentially toxic elements in mantle, digestive gland, and branchial hearts of *Octopus hubbsorum* in relation to the total concentration in whole clams *Megapitaria squalida*. All data are from two sites near Santa Rosalía and one in La Paz Bay, Gulf of California. The highest values are highlighted in bold.

Tissue	Locality	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn
Mantle	Santa Rosalía A	0	0.4	1.7	0.1	0.4	0	0	3.2
	Santa Rosalía B	0	0.3	2.6	0	0.4	0	0	2.1
	La Paz Bay	0	0	2.2	0	0.2	0	0	1.6
Digestive gland	Santa Rosalía A	73	31	**250**	2.3	4	1	30	46
	Santa Rosalía B	50	22	**300**	1.8	7.2	0.8	13	32
	La Paz Bay	5.4	2.5	**225**	2.2	1.3	0.8	24	20
Branchial hearts	Santa Rosalía A	38	**203**	7.2	11	15	**233**	0	7.7
	Santa Rosalía B	32	**137**	10	3.1	15	**132**	0	4.2
	La Paz Bay	1.3	10	9.6	3.0	2.4	57	0	4.0

cephalopods, Zn, Cu and Cd are associated with both high and low molecular weight proteins in digestive gland while Pb is associated with unknown high molecular weight proteins (Bustamante et al., 2006; Raimundo et al., 2010b). Cytosolic proteins include metallothioneins, which are known to bind Cd, Cu, Hg and Zn (Marigómez et al., 2002). Furthermore, the high Cu and Zn levels are related to their role as essential elements involved in various metabolic processes, such as the formation of metallo-dependent enzymes involved in digestion (Craig and Overnell, 2003). For its part, Pb was in lower concentrations than Cu, Cd and Zn and this could be due to its possible elimination though other excretion pathways (nephrons, heart and intestine), as has been described for molluscs (Marigómez et al., 2002).

The high concentrations of Co > Ni > Mn > Sr > Al > V > Cr in the branchial hearts of *O. hubbsorum* from these Gulf of California sites relative to all other tissues confirms that, although this organ represents a mere 0.2% of total weight, it is nonetheless an important site for the storage and clearance of elements (Mangold et al., 1989; Miramand and Fowler, 1998). The bioaccumulation of these specific elements in branchial hearts of octopuses is likely because 37% of the dry weight of this organ is composed of adenochromes (Fox, 1976), which are pigments with a high affinity for Fe (Ito et al., 1976), Co (Nakahara and Shimizu, 1985) and V (Miramand and Guary, 1980), and possibly other elements, and are considered as key molecules for their storage and clearance. Other elements, such as Al and Sr, may be up taken in gills, as has been reported in fishes (Schiffman, 1961; Exley et al., 1991), and retained by the branchial hearts through the filtration of blood from the gills (Mangold et al., 1989); however, the role of branchial hearts in storage and clearance of Al and Sr remains unknown. Along these lines, the fact that the concentrations of PTEs in *O. hubbsorum* gills were not higher relative to other tissues suggests that the former accumulates elements over the short term, as reported in bivalve molluscs such as *Ruditapes decussatus* (Bebianno and Serafim, 2003) and *Cerastoderma glaucum* (Machreki-Ajmi et al., 2008).

The low PTE concentrations in the mantle of *O. hubbsorum* relative to all other tissues is consistent with the findings reported for *O. vulgaris*

Table 5
Concentrations of potentially toxic elements (mean ± SD) in some bivalves that represent potential prey of *Octopus hubbsorum* in Santa Rosalía and La Paz Bay, Gulf of California.

Potential preys	Locality	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Megapitaria squalida	Santa Rosalía A	1.1 ± 0.1	1.7 ± 0.1	14.6 ± 1.8	122 ± 14	3.7 ± 1.4	1.26 ± 0.09	0.24 ± 0.03	19 ± 1.2	Present study
	Santa Rosalía B	1.3 ± 0.06	2.0 ± 0.1	9.5 ± 0.9	193 ± 5	2.8 ± 0.06	1.7 ± 0.04	0.3 ± 0.03	25 ± 1.4	
	La Paz Bay	9.7 ± 0.1	2.3 ± 0.06	9.3 ± 0.4	205 ± 3	4.7 ± 0.1	3.2 ± 0.2	0.1 ± 0.01	40.1 ± 0.9	
Mytilus edulis	Santa Rosalía A	4.0 ± 0.4		49.6 ± 9.8	369 ± 125	0.5 ± 0.1	7.8 ± 0.6	5.8 ± 0.4	47.9 ± 3.4	Cadena-Cárdenas et al., 2009
	Santa Rosalía B	3.5 ± 0.2		17.1 ± 3.8	150 ± 24	0.8 ± 0.2	6.4 ± 0.7	4.8 ± 0.5	52.9 ± 4.8	
Modiolus capax[*]	Santa Rosalía	15[a] 20[b]		58.6[a] 89[b]		500[a] 900[b]			160[a] 320[b]	Gutiérrez-Galindo et al., 1999

[*] Average concentration by total length: a = large clams (98.6 mm), b = medium clams (75.5 mm).

Table 6
International standards for some potentially toxic elements (As, Cd, Cr, Cu, Hg, Ni and Pb) in edible tissues of fish, molluscs, bivalves and cephalopods for human consumption.

Element	Organisms	Standards (mg kg^{-1}, dry weight)	Reference[a]
As	Molluscs	86.0	FDA
		80.0	NOM 2009
Cd	Fish	1.3	EC
	Molluscs	2.0	NOM 2009
		4.0	FDA
	Bivalves	5.0	EC
		2.0	CODEX
	Cephalopods	2.5	NOM 1995
		2.0	CODEX
Cr	Molluscs	13.0	FDA
Cu	Bivalves	32.5	FDA
Hg	Fish	5.0	EC
	Cephalopods	5.0	NOM 1995
Ni	Molluscs	80	FDA
Pb	Fish	2.5	NOM 2009
		1.5	EC
		1.5	CODEX
	Molluscs	1.7	FDA
		1.0	NOM 2009
	Bivalves	1.5	EC
	Cephalopods	1.0	NOM 1995
		1.0	EC

[a] CODEX: CODEX STAN 193-1995 Amended in 2015 (CODEX FAO/WHO, 2015).
EC: European Commission (EC) No 1881/2006 (Commission, 2006).
FDA: US FDA Guidance documents (US-FDA, 2007).
NOM 1995: NOM-129-SSA1-1995 (DOF, 1996).
NOM 2009: NOM-242-SSA1-2009 (DOF, 2011)

(Miramand and Guary, 1980; Napoleão et al., 2005; Semedo et al., 2012) and other cephalopods such as *Sepia officinalis* and *Loligo vulgaris* (Rjeibi et al., 2014). This is likely because the muscle tissues are not active sites of biotransformation and accumulation of PTEs (Elnabris et al., 2013). The mantle cannot store PTEs over the long term because it lacks of specific binding proteins (e.g. metallothioneins). In addition, some elements are transferred from the mantle through the blood stream to the branchial hearts and digestive gland (Bustamante et al., 2002).

4.3. Differences in PTE bioaccumulation between sites

The concentration of PTEs in *O. hubbsorum* tissues were similar in Santa Rosalia sites A and B, despite the different contamination levels recorded in the sediments (Shumilin et al., 2011, 2013). This may be attributed to the fact that octopuses are constantly moving between areas to find food and shelter according to predator pressure (Mather, 1982). For example, *O. vulgaris* can travel up to 50 km in 40 days in search of its preferred prey (Itami, 1964), while the distance between the Santa Rosalia sites is just 2 km.

When compared to La Paz Bay, octopus tissues from Santa Rosalia were higher in almost all of the same elements found at higher concentrations in the sediments from this port, namely Mn > Cu Zn > Pb > Co > U > Cd (Shumilin et al., 2013); the exceptions were Cu and U, which occurred at similar concentrations in all octopus tissues in Santa Rosalia and in La Paz Bay, and Zn, which only showed higher concentrations gills of octopus from Santa Rosalía.

However, significant differences in elemental concentrations were found in all tissues except the mantle (Cd, Co, Cr, Mn and Ni in branchial hearts; Cd, Cr, Mn and Zn in gills; Co, Mn, and Pb in digestive gland). This highlights the high PTE clearance capacity in octopus, because even when these organisms are exposed to elevated PTEs, their concentrations in muscle tissue (mantle) are still low as has been reported in other octopus species (Napoleão et al., 2005; Raimundo and Vale, 2008).

Mn is an essential element (Newman, 2014) commonly found at high concentrations in the digestive gland and branchial hearts of octopus species (Napoleão et al., 2005). However, in *O. hubbsorum* from Santa Rosalia, Mn levels were up to five times higher than octopus from La Paz Bay in those same tissues, and almost 10 times higher than those reported for other octopus species (Table 3). Similarly, the concentrations of Co found in branchial hearts of *O. hubbsorum* from Santa Rosalia exceeded -by up to 50 times- the levels recorded for this organ in other species of octopus, squids and in bivalve tissues (Yoshida, 1981; Nessim and Riad, 2003). This is further evidence of the effect of mining wastes in the study area.

The octopuses from Santa Rosalia also showed an additional physiological compensation related to mining wastes, because in this site they showed higher concentrations of Co and Cd in branchial hearts than in their digestive glands, even when it is known that the storage and clearance of these elements take place primarily in the digestive gland (Miramand and Bentley, 1992). This suggests that the uptake capacity of the digestive gland was exceeded, and that hearts serve as ancillary clearance organs (Bustamante et al., 1998b).

Although Cu, Zn and U are also among the most concentrated elements in Santa Rosalia sediments, their concentrations in tissues of *O. hubbsorum* did not reflect this pollution. This can be attributable to the fact that Cu and Zn are essential elements whose concentrations are regulated by various homeostatic mechanisms. Both elements are involved in the synthesis of several metallo-dependent enzymes (Craig and Overnell, 2003). Cu is mainly used in the synthesis of hemocyanin, a respiratory pigment containing 0.25% copper (Bustamante et al., 2000), as well as in the production of amine oxidase, a toxic component present in the salivary glands of octopuses (Nessim and Riad, 2003; Raimundo and Vale, 2008). In addition, it has been reported that many species of crustaceans and molluscs maintain a relatively constant total body load of Cu despite wide environmental concentrations (Taylor and Anstiss, 1999) and this could be the case for *O. hubbsorum*. However, to be able to better explain this exception, studies on the physiology of Cu in this species would be necessary. For its part, the lack of differences in

U concentrations between octopuses from Santa Rosalia and La Paz Bay suggests that this element is available at similar concentrations in both sites, possibly due to the natural U deposits in La Paz (SGM, 2014).

Although La Paz Bay is not considered an area highly polluted by PTEs (Rodríguez-Castañeda et al., 2006), octopuses from this site had higher Ag, Cr, Ni and Sr in the digestive glands and higher As and Hg in all tissues when compared to results from Santa Rosalia. This finding confirms that As clearance does not occur mainly in the digestive gland of cephalopods and it has a high affinity for muscle tissue in the mantle and arms (e.g. Napoleão et al., 2005; Seixas et al., 2005b; Semedo et al., 2012). However, the high As in octopus tissues from La Paz Bay were in contrast to the low As in sediments and algae from this location (Rodríguez-Castañeda et al., 2006). A plausible explanation involves the diet, because if octopuses from La Paz Bay consume larger quantities of crustaceans relative to those from Santa Rosalia, this could lead to higher As concentrations in the tissues of the former, as has been suggested for *N. macromphalus* (Bustamante et al., 2000).

With respect to Hg, octopus accumulates this element primarily from the diet, so it is stored and cleared by the digestive gland (Seixas et al., 2005b; Rjeibi et al., 2014). Foodborne Hg in cuttlefish, squid (Rjeibi et al., 2014) and bivalves (Marigómez et al., 2002) is commonly transferred through the digestive gland and stored in muscle tissue; however, in some bivalves aqueous Hg accumulates both in the mantle epithelial cells and in gills (Marigómez et al., 2002). Accordingly, the high Hg concentrations in the mantle and branchial hearts of *O. hubbsorum* from La Paz Bay could be due to high Hg concentrations in water and its direct uptake through the epithelium and gills, respectively. However, there are no studies available reporting aqueous Hg concentrations in La Paz Bay.

4.4. Biomagnification of PTEs

At all sites, *O. hubbsorum* had higher concentrations of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn in its tissues relative to the concentrations of the same elements in one of its prey (the clam *M. squalida*), indicating biomagnification. As in other octopus species, *O. hubbsorum* feed upon bivalve molluscs (López-Uriarte et al., 2010), which usually have high PTE concentrations due to their direct exposure to the substrate, filter-feeding, and tolerance to high pollutant levels, among others (Jakimska et al., 2011). However, molluscs account for just 30% of the octopus diet (López-Uriarte et al., 2010) therefore future research should consider other prey such as crustaceans (± 60% of the diet) (López-Uriarte et al., 2010) for a more comprehensive assessment of PTE biomagnification in this species.

4.5. PTEs standards for human consumption of octopus

The mean concentrations of PTEs in the mantle of *O. hubbsorum* collected in Santa Rosalia and La Paz Bay do not pose a public-health risk, according to international standards for human consumption (Table 6). Among the tissues examined, only the mantle is consumed frequently by humans. However, the digestive gland and/or branchial hearts of octopus from Santa Rosalia or La Paz Bay exceeded the Cd, Cu, Ni and Pb levels established in the standards mentioned above (Figs. 2 and 3); hence these organs are not suitable for consumption.

5. Conclusions

This study presents the first data on PTEs in octopuses from the Gulf of California, and provides evidence of high bioaccumulation and biomagnification of PTEs in octopuses from sites known to have historical metal contamination. Nevertheless, *O. hubbsorum* show the ability to regulate the high concentrations of most PTEs through specific organs (digestive gland and branchial hearts) maintaining similar elemental composition of the mantle among sites and levels below PTE standards for human consumption of octopus.

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