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Heavy Metals in the Clam *Megapitaria squalida* Collected from Wild and Phosphorite Mine-Impacted Sites in Baja California, Mexico

Considerations for Human Health Effects

Lia Méndez,* Elena Palacios, Baudilio Acosta, Pablo Monsalvo-Spencer, and Ticul Alvarez-Castañeda

Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Mar Bermejo 195, Playa Palo Santa Rita, La Paz, B.C.S. 23090, Mexico

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ABSTRACT

The "chocolate clam" *Megapitaria squalida,* is widely consumed by the population of several localities along the Pacific coast. Clams collected from seven stations in Bahía de la Paz, a bay within the Gulf of California, before and after the summer rainy season were analyzed for Pb, Ni, Cd, Mn, Zn, Cu, and Fe. The location of the sampling sites significantly affected the concentration of metals in clam tissues, but not in relation to the proximity to alleged contaminated sites. Clams from a site close to a phosphate mine had the highest levels of Pb, but only in April, and the highest concentrations of Cd were recorded in clams collected in areas with no anthropogenic activities. Clams from sites considered clean had higher levels of Cd, Fe, Zn, and Mn. The mean concentrations (μg/g dry weight) ranged from 0.1 to 7.8 for Pb, from 1.9 to 8.8 for Ni, from 1.5 to 11.1 for Cd, from 2.5 to 14.1 for Mn, from 47.2 to 64.6 for Zn, from 5.4 to 18.7 for Cu, and from 154 to 558 for Fe. Collecting clams in sites apparently pristine is no guarantee that metals will be in low concentrations.

Index Entries: Heavy metals; bivalves; clam; contamination; mollusk; nutrition; toxicology.

* Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Bivalves are important commercial seafood worldwide, but being mostly sessile, they cannot escape polluted waters. Mussels, clams, and oysters have high levels of essential long-chain fatty acids and, contrary to shrimp, they have lower levels of cholesterol, as they accumulate phytosterols; thus, they are considered a healthier alternative to crustaceans. Their sedentary existence makes them useful biomonitors of heavy metal pollution in coastal water. Other characteristics that make them desirable as biomonitors are their filtering habits, residence in shallow water (hence easy to sample), long life span and high survival rate, and ease of identification *(1,2)*. The same characteristics that make them good metal pollution indicators can also turn them into potentially dangerous seafood. Elements such as Cd, Cu, Pb, and Zn are widely distributed in coastal environments *(2)*, where bivalves usually grow. However, metal accumulation depends on the proportion of these elements in a bioavailable form *(3,4)*, which is, in turn, influenced by pH, oxygen, salinity, temperature, and organic matter *(5)*. Numerous studies have quantified the content of heavy metals in several mollusk species *(2,3,6–8)* and concluded that the accumulation of heavy metals in these shell fish is influenced by the season of sampling, size, sex, and hydrodynamics of the environment *(4,9)*.

La Paz Bay, Baja California South, Mexico, has been traditionally characterized as free of industrial sources of pollution and having a minimal anthropogenic impact because of low population density and tourist activities. However, for several years, the municipal water treatment plant unloaded partly treated and sometimes untreated sewage into the lagoon at the south of the bay. Additionally, about 50 km north of the city of La Paz, a phosphate mine had been active for 25 yr until operations stopped in 2001 after a hurricane destroyed part of their installations. During the summer rainy season, several elements derived from soil, weathered rock, dumping in drainage channels, untreated and partially treated sewage, and urban flood water are washed into the bay.

Most countries have guidelines for permissible levels of heavy metals in food *(2)*. Because guidelines for each species is impractical, the amount of metals consumed per gram of meat must be similar and species independent. In this study, we used the chocolate clam *(Megapitaria squalida)*, a common coastal clam found from the central coast of the Baja California Peninsula of Mexico to northern Peru *(10)*. The chocolate clam is frequently consumed fresh and cooked, and its sales make up an important economic input along the Pacific coast. The objective was to evaluate the consumption of heavy metals per meal as related to the season and place of sampling.

MATERIALS AND METHODS

Seven sites were sampled near the shore of the La Paz Bay (B.C.S., Mexico) in April (dry season) and October (rainy season) of 2003 (Fig. 1).

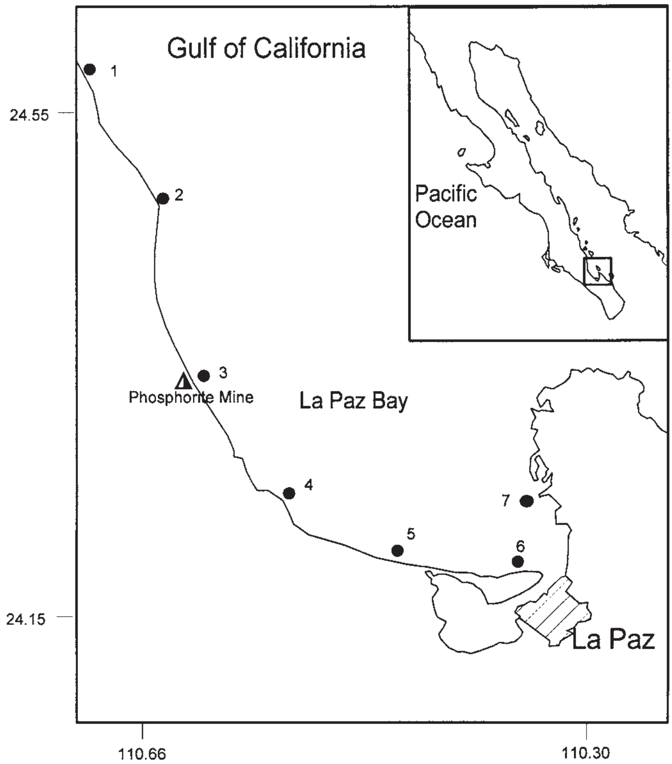

Fig. 1. Location of sampling sites in La Paz Bay, B.C.S. Mexico.

A phosphate mine that had been active for 25 yr is located in front of site 3. Site 6 is located near the mouth of the Ensenada de La Paz, a lagoon separated from the bay by a large sandbar. The city of La Paz is located in this lagoon. During the rainy season, sediment and debris are washed into the sea via several arroyos (dry stream beds) draining the surrounding mountains, alluvial fans, and coastal plain. From each site, 10 clams of the *Megapitaria squalida* species were collected at a depth ranging from 6 to 10 m. Each clam was measured, weighed, and dried in an oven at 70°C until a constant weight was recorded (approx 36 h). Individual clams were then digested in acid-washed Teflon tubes with concentrated nitric acid in a microwave oven (CEM model Mars 5X; Matthews, NC). The cooled, dry samples were dissolved in 1 mL of concentrated HCl and 24 mL of deionized water in a volumetric flask *(8)*. Samples were analyzed by atomic absorption (AVANTA; GBC Scientific Equipment, Dandenong, Australia) using an air–acetylene flame. Certified standard reference material TORT-2 (National Research Council of Canada, Ottawa) was used to check accuracy. Analytical values were within the range of certified values. Recovery of the metals was over 95%. The amount of each metal per clam during a meal is expressed in wet weight. The shells of *Megapitaria squalida* do not have homogeneous growth. To make objective comparisons between specimens of different sizes, a morphometric index proposed by Méndez et al. *(8),* based on morphometric characteristics of each clam, was used. A higher index indicates a lower size.

Bifactorial analyses of variance (ANOVAs) were made to compare the biometry of clams in the bay, using sites as the first independent variable (S = 7 levels) and the month of April and October as the second independent variable (M = 2 levels); results of the ANOVA are shown in the last three columns of Tables 1 and 2. A Tukey post hoc test compared individual means, where different letters indicate a significant difference ($p <$ 0.05). Multiple regression analysis between the size of clams and the amount of heavy metals was performed. All statistical methods used STATISTICA, version 5.0 (StatSoft, Inc., Tulsa, OK, USA). Results are reported as means ± standard error.

RESULTS

The morphometric data for clams sampled at each site are shown in Table 1. The size of the clams was sampling-site dependent, and differences in shells lengths were recorded before and after the rainy season (p < 0.05). Significant correlations show that at each sampling site, the size was different depending on the sampling season. As shown by the morphometric index, only the clams sampled from site 7 and those from site 2 sampled in October were significantly smaller than the clams collected at the other sites during the dry and rainy seasons.

All heavy metal levels were affected by the sampling site and season (Table 2). The metal concentration in clams decreased ($p < 0.05$) in the following order: Fe > Zn > Cu > Mn > Cd ≈ Ni > Pb. There were two orders of magnitude difference between concentrations of Fe and Pb. The rainy season had a significant effect on the concentration of metals in clams. The highest concentration of Cd was found in clams sampled at site 2 in the dry season and the lowest at site 7 at both seasons. The highest concentration of Ni was found in clams sampled at site 6 (Fig. 1) in the rainy season and the lowest at site 5 in the dry season. Cu was highest at sites 5 and 6 in the rainy season. Mn was highest at sites 1 and 5 in the rainy season and lowest in general in the dry season. Pb was highest at site 4 in the dry season. Fe was highest at sites 4 and 6 and lowest at site 7 in the rainy season. Zn was highest at sites 4 and 5 in the dry season.

Several correlations were found between the levels of metals in each clam that were significant before and after the rainy season: Cd–Fe, Ni–Fe, Mn–Fe, Mn–Zn, and Fe–Zn (Table 3). Other correlations were only found before the rainy season (April), but not after (October): Cd–Cu, Ni–Mn, and Cu–Zn. There was a significant inverse correlation between Ni and Pb before the rainy season (April). After the rainy season, there were some significant correlations that were not observed before the rainy season: Cd–Ni, Cd–Mn, Cd–Zn, and Ni–Zn.

Table 1
Biometry of *Megapitaria squalida* Collected in Seven Sites (S) Before (April) and After (October) the Rainy Season (M) at La Paz Bay

Station		1	2	3	4	5	6	7	S	M	S M
Total weight[A]	April	123 ± 6^{ab}	101 ± 3^{ab}	116 ± 9^{ab}	94 ± 3^{ab}	102 ± 8^{ab}	116 ± 7^{ab}	167 ± 10^{a}	**	N.S.	*
	October	97 ± 3^{ab}	124 ± 9^{ab}	109 ± 3^{ab}	108 ± 7^{ab}		115 ± 7^{ab}	147 ± 11^{a}			
Length (cm)	April	79.0 ± 1.20^{ab}	78.0 ± 0.9^{ab}	80.4 ± 1.9^{ab}	75.3 ± 0.8^{b}	75.9 ± 1.7^{b}	79.7 ± 1.2^{ab}	98.5 ± 5.9^{a}	**	*	*
	October	75.1 ± 0.8^{b}	85.9 ± 3.4^{a}	79.1 ± 0.7^{ab}	78.1 ± 1.8^{ab}	74.9 ± 1.1^{b}	80.7 ± 1.1^{ab}	93.5 ± 3.0^{a}			
Width (cm)	April	66.0 ± 0.6^{b}	61.8 ± 0.5^{bc}	64.3 ± 1.8^{bc}	57.6 ± 2.5^{c}	60.9 ± 1.2^{bc}	65.4 ± 1.0^{b}	80.5 ± 1.9^{a}	**	N.S.	**
	October	61.4 ± 0.8^{bc}	74.3 ± 1.9^{a}	64.2 ± 0.9^{bc}	63.6 ± 1.5^{bc}	60.7 ± 1.0^{bc}	$66.7 \pm .1.1^{b}$	76.6 ± 1.9^{a}			
Height (cm)	April	41.3 ± 0.7^{ab}	39.4 ± 0.4^{ab}	40.5 ± 0.9^{ab}	36.2 ± 0.3^{b}	38.5 ± 1.0^{b}	40.8 ± 0.7^{ab}	47.8 ± 1.2^{a}	**	N.S.	**
	October	38.3 ± 0.5^{b}	43.4 ± 1.2^{ab}	39.3 ± 0.4^{ab}	39.3 ± 1.1^{ab}	37.2 ± 0.6^{b}	39.6 ± 0.9^{ab}	44.8 ± 1.3^{a}			
Tissue weight (g)	April	24.8 ± 0.9^{b}	24.5 ± 1.2^{b}	25.7 ± 2.1^{b}	23.1 ± 0.8^{b}	23.5 ± 1.4^{b}	24.8 ± 1.4^{b}	44.2 ± 2.5^{a}	**	N.S.	*
	October	19.9 ± 1.4^{b}	36.9 ± 2.5^{ab}	21.0 ± 1.1^{b}	22.1 ± 1.5^{b}	19.5 ± 1.1^{b}	23.3 ± 1.0^{b}	50 ± 5.2^{a}			
Index[B]	April	7.6 ± 0.2^{a}	7.4 ± 0.3^{a}	7.5 ± 0.5^{a}	7.4 ± 0.3^{a}	7.6 ± 0.3^{a}	7.7 ± 0.4^{a}	5.2 ± 0.2^{b}	**	*	*
	October	9.0 ± 0.4^{a}	5.6 ± 0.2^{b}	8.9 ± 0.4^{a}	8.4 ± 0.4^{a}	9.1 ± 0.4^{a}	8.1 ± 0.3^{a}	4.6 ± 0.4^{b}			

Note: N.S. = not significantly different, N.S. > 0.05; $*0.05 \geq p \geq 0.01$; $**p < 0.01$. Different letters (a, b, c) indicate significant ($p < 0.05$) differences between the measurements of each parameter.

[A] Total weight = wet tissue + shell.

[B] Morphometric index = (length + height + width)/tissue wet weight *(8)*.

Table 2
Metal Concentrations (μg/g Dry Tissue Weight) in *Megapitaria squalida* Collected in Seven Sites (S) Before (April) and After (October) the Rainy Season (M) at La Paz Bay

Site		1	2	3	4	5	6	7	S	M	S M
Pb	April	2.5±0.8^{ab}	0.3±0.2^{b}	4.8±0.5^{bc}	7.8±1.9^{c}	5.2±0.9^{bc}	3.6±0.7^{ab}	2.0±0.5^{ab}	*	**	**
	October	2.6±0.7^{ab}	2.4±0.7^{ab}	1.5±0.6^{b}	0.2±0.01^{b}	0.1±0.05^{b}	1.2±0.4^{b}	0.8±0.6^{b}			
Ni	April	5.1±0.6^{ab}	5.2±0.6^{ab}	4.4±0.4^{ab}	3.6±0.6^{ab}	1.9±0.4^{b}	5.0±0.8^{ab}	6.4±0.6^{ab}	**	**	**
	October	5.5±0.7^{ab}	4.6±0.4^{ab}	6.2±0.5^{ab}	5.8±0.4^{ab}	4.8±0.4^{ab}	8.8±1.0^{a}	3.9±0.8^{ab}			
Cd	April	7.0±0.6^{ab}	11.1±1.2^{a}	5.2±0.4^{ab}	6.1±0.7^{ab}	8.3±0.6^{ab}	4.5±0.5^{ab}	1.8±0.1^{b}	**	**	**
	October	7.0±1.0^{ab}	3.2±0.3^{ab}	5.8±0.5^{ab}	5.8±0.8^{ab}	4.1±0.5^{ab}	6.6±0.6^{ab}	1.5±0.7^{b}			
Mn	April	3.3±0.7^{b}	4.3±0.6^{b}	2.8±0.5^{b}	4.7±0.5^{b}	4.1±0.6^{b}	2.5±0.4^{b}	6.3±0.8^{ab}	**	**	**
	October	11.0±1.0^{a}	6.0±0.7^{ab}	6.0±0.3^{ab}	9.7±1.4^{ab}	14.1±1.0^{a}	9.3±0.8^{ab}	5.9±2.7^{ab}			
Zn	April	49.5±1.9^{b}	55.1±4.0^{ab}	52.3±1.4^{ab}	62.9±2.1^{a}	64.6±2.74^{a}	51.2±2.4^{ab}	57.8±2.9^{ab}	**	**	**
	October	49.7±2.4^{b}	47.7±1.9^{b}	54.1±2.3^{ab}	55.7±2.8^{ab}	48.5±1.8^{b}	51.4±2.5^{ab}	47.2±2.8^{b}			
Cu	April	7.1±0.4^{b}	7.5±0.6^{ab}	5.4±0.3^{b}	8.0±0.4^{ab}	7.5±0.5^{ab}	5.9±0.3^{b}	5.8±0.3^{b}	*	*	*
	October	5.5±0.3^{b}	6.5±0.5^{b}	5.5±1.5^{b}	5.7±0.5^{b}	18.4±6.1^{a}	18.7±6.1^{a}	11.5±3.1^{ab}			
Fe	April	258±15^{ab}	385±31^{ab}	276±13^{ab}	323±56^{ab}	295±26^{ab}	278±15^{ab}	344±22^{ab}	**	**	**
	October	433±218^{ab}	259±30^{ab}	438±39^{ab}	548±49^{a}	471±46^{ab}	558±70^{a}	154±19^{b}			

Note: N.S. > 0.05; *$0.05 \geq p \geq 0.01$; **$p < 0.01$. Different letters (a, b, c) indicate significant ($p < 0.05$) differences between the measurements of each element.

Table 3
Correlations Between Metals (µg/g Dry Tissue Weight) in Tissues of *Megapitaria squalida* Collected Before (April) and After (October) the Rainy Season at La Paz Bay

		April						
		Pb	Ni	Cd	Mn	Zn	Cu	Fe
O	Pb		0.07	0.07	0.014	0.18	-0.16	-0.13
c	Ni	**-0.36**		**0.44**	**0.24**	0.002	-0.18	**0.33**
t	Cd	-0.07	-0.07		0.09	0.15	**0.29**	**0.41**
o	Mn	-0.12	0.23	**0.25**		**0.48**	0.21	**0.32**
b	Zn	-0.03	**0.60**	**0.36**	**0.33**		0.001	**0.40**
e	Cu	0.06	0.19	0.01	0.144	**0.45**		0.08
r	Fe	-0.12	**0.44**	**0.57**	**0.42**	**0.30**	0.14	

Note: The correlation in bold is significant ($p < 0.05$).

Table 4
Correlations Between Metals (µg/g Dry Tissue Weight) and Morphometric Variables of *Megapitaria squalida* Collected Before (April) and After (October) the Rainy Season at La Paz Bay

	Month	Width (cm)	Height (cm)	Total Length (cm)	Weight (g)	Tissue weight (g)
Pb	April	**-0.25**	-0.20	**-0.32**	-0.21	-0.22
	October	-0.03	-0.10	-0.04	-0.10	-0.06
Ni	April	**0.50**	**0.42**	**0.46**	**0.49**	**0.44**
	October	-0.03	-0.13	-0.03	0.08	-0.18
Cd	April	**-0.49**	**-0.46**	**-0.39**	**-0.44**	**-0.50**
	October	**-0.30**	**-0.38**	**-0.25**	**-0.26**	**-0.41**
Mn	April	**0.32**	0.21	0.20	0.20	**0.31**
	October	**-0.46**	**-0.55**	**-0.55**	**-0.48**	**-0.42**
Zn	April	-0.04	-0.13	-0.17	-0.12	0.01
	October	-0.18	**-0.24**	**-0.23**	-0.17	-0.21
Cu	April	**-0.39**	**-0.40**	**-0.40**	**-0.36**	**-0.31**
	October	0.13	0.09	0.07	0.19	0.09
Fe	April	0.14	0.09	0.08	0.01	0.04
	October	**-0.33**	**-0.49**	**-0.35**	**-0.28**	**-0.52**

Note: The correlation in bold is significant ($p < 0.05$).

When the correlations were made between levels of heavy metals and morphometric variables of clams sampled before and after the rainy season, we found significant but negative correlations between Cd and all morphometric variables measured (Table 4). Ni was positively correlated with morphometric variables. Cu was negatively correlated, but only before the rainy season. Mn was positively correlated to tissue weight and shell length before the rainy season and negatively correlated to all morphometric variables after the rainy season. Pb was negatively correlated to shell length and thickness but only before the rainy season. Fe was negatively correlated to all morphometric variables after the rainy season. Zn was negatively correlated to shell width and height after the rainy season.

DISCUSSION

In comparison with several guidelines on heavy metal for food safety set by different countries, some areas in our study show higher concentrations than the permissible limits set by the FAO *(11)* and by the Malaysian Food regulation *(2)* for Cd (1.00 μg/g wet weight) and Pb (2.00 μg/g wet weight). Some of these areas (Table 2) are considered pristine. The remaining metals analyzed in this study are below the levels reported as normal *(2,8,11)*.

Rains affect the input of organic material from rivers *(4)* and the salinity of the water column that has an influence on the bioavailability of some heavy metals in sediments *(12)* and, in turn, this could affect the heavy metal concentrations in clams. For example, Otchere *(4)* found a higher level of Fe in clams collected during the rainy season. We did find the highest concentrations of Cu in October, after the rainy season. Cu contamination can increase as a result of wastewater from agriculture *(13,14)*. Thus, an increase in Cu levels during the rainy season was expected as a result of the introduction of terrine materials from agricultural areas to the sea, especially during hurricanes.

We did not expect differences in the amount of terrine material introduced into the sea among sites in the rainy season because all sites are near small streams with floodwater present only after large rains. However, the composition of the sediment can change from one place to another. In effect, the concentrations of metals in clam tissues depended strongly on sampling location. Site 3, located in front of a phosphate mine, was expected to show the highest levels of metals in clam tissues, such as Cd, Ni, and Zn, because these metals are found associated to phosphorite and probably dumped into the sea during mining operations *(14)*. We did find higher levels of Pb and Zn in front of the mine and in the closer sites (sites 4 and 5) but only during the dry season (April), after winter, during which the currents transport water enriched with nutrients from upwelling areas. We did expect high levels of Pb at sites 5, 6, and 7 because they are relatively close to La Paz, where previous studies *(15)* reported elevated concentrations of Pb in sediments, probably caused by gasoline used before the 1990s by boats, especially in areas used for yachts and vessel anchorage. However, Pb was found in high levels in clams sampled in two of the three areas considered pristine (sites 1 and 2). Other sources of metals could be phosphorite deposits along the peninsula, some of them in the north of our area of study that can be transported by currents to La Paz Bay *(16)*.

Nickel was highest in clams sampled at site 6, but only during the rainy season (October). Ni in the environment is associated with the petrochemical industry; in agreement, gasoline deposits are located close to site 6. Ni can also be concentrated as a result of inadequate battery disposal and seep into the water supply and, from there, to the sea, but in this case, we would expect high levels of Cd, which is the most toxic component of Cd-Ni batteries. This significant correlation occurs during the rainy season (Table 3; $r = 0.44$; $p < 0.05$). However, we did not find high levels of Cd at site 6. Cd might not be absorbed at the same rate as Ni by clams, possibly because bioavailability is lower. In contrast, the highest concentration of Cd in October was found at sampling site 1 and the highest levels of Cd and Fe in April were found at site 2. Both areas were, at least in appearance, considered pristine because of very low anthropogenic influence and far from any expected source of contamination. High levels of Cd might originate from the San Gregorio Formation. This formation is one of the two largest rock phosphate deposits in the world *(5)* and is reported to have elevated Ca and P levels, with Cd as a common impurity *(17,18)*. Several works have reported anomalous levels of Cd in plankton *(19)*, sea skaters *(20)*, and sediments near this site *(15)*. Cd levels at this site are apparently of geological origin and not a result of human activities. High levels of Cd in mussels are also related to upwelling areas *(21)*

The popular notion that contamination-free sites are located far from human or industrial activities is not supported by the present results. The question that arises is, "Can clams that are sampled from "pristine" areas and that might have a high metal content be safely consumed?" In terms of human health, heavy metals are divided into two groups: those whose levels have to be kept to a minimum, such as Pb and Cd, and those that are essential at low doses but can be harmful at higher concentrations. An adult can consume 200 g of chocolate clam per day. Considering, for example, Pb, the JECFA *(22)* established a provisional tolerable weekly intake of 25 μg/kg of body weight (equivalent to 3.5 μg/kg of body weight per day). On average, Pb levels calculated in relation to 200 g wet weight of clam meal (Table 5) fall below the permitted levels for human consumption, but are, nonetheless, high in April. However, few people eat chocolate clams daily.

In relation to Ni, a 200-g clam meal has approx 204 ± 11 μg, 33% lower than the maximum tolerable daily intake (Table 5). The levels of Ni found in clams in the present study are comparable to the levels found in clams collected in pollution-free areas *(8)* and in contrast to levels of Ni found in clams (up to 43 μg/g) collected close to petrochemical plants *(7)*. Cd levels calculated in the same 200 g of clam tissue are almost three times higher (Table 5) than the maximum value recommended by WHO *(23)*. Shellfish contain naturally elevated amounts of Cd. Individuals who consume large amounts of seafood might at first seem to be at increased risk. However, recent studies have demonstrated that foods that are naturally enriched in Cd are also enriched in substances, such as metallothioneins, that inhibit the uptake of Cd into the body *(24)*.

Table 5
Metal Levels (µg) in 200 g of Fresh Clam Tissue: Comparison to Recommended Daily Ingestion (µg) and Maximum Tolerable Daily Intake Concentrations (µg) for Each Metal

	April	October	Recommended Daily	Maximum Tolerable
Pb	149 ± 17	51 ± 9	--	245[a] (22)
Ni	181 ± 10	227 ± 11	--	350[a](23)
Cd	251 ± 16	203 ± 13	--	70[a](23)
Mn	160 ± 10	357 ± 22	3,800 (35)	11,000 (36)
Zn	2,249 ± 45	3,285 ± 60	15,000 (35)	70,000[a](23)
Cu	269 ± 7	404 ± 56	2,500 (35)	35,000[a](23)
Fe	12,338 ± 466	16,573 ± 889	18,000 (35)	56,000[a] (23)

Note: The "a" following the maximum tolerable concentration indicates that it is based on a person's body weight of 70 kg.

In relation to essential metals, the recommended daily requirement of Fe is between 10 and 50 mg *(23).* The concentration of Fe in clams from La Paz Bay was approx 0.4 ± 0.1 g/kg of clam. Therefore, a 200-g clam meal would provide approx 14.4 ± 6.7 g of Fe, which is approx 80% of the daily dose range recommended of Fe. For Cu, the values for a 200-g clam meal is approx 336 ± 51 µg, which is about 13% of the dose recommended for a 70-kg, adult whereas for Mn and Zn, it is almost 7.4% 18%, respectively, of the daily requirement for adults.

Simkiss et al. *(6)* proposed that some correlations between metals in mollusks are so close that deviations from this relationship can be used as an index of pollution. Frazier *(25)* found correlations between some metals, Mn–Fe, Fe–Zn, and Cu–Cd, in oyster. We found a significant correlation for Mn–Fe and Fe–Zn in both seasons, but for Cu–Cd, it was found only in the dry season. Also, in oysters, Hugget et al. *(26)* found a correlation between Cu and Zn at different salinities. We did find a correlation between Cu and Zn (Table 3; $r = 0.45$; $p < 0.05$), but only in the dry season (April). The absence of a correlation in October could be a result of a very high concentration of Cu during this month, but only at sites 4, 5, and 6, near La Paz.

Some variations found in relation to metal levels might be a result of size and age of the organisms. Most studies have established a negative relationship between Zn, Cd, Fe, Pb, and Cu and the size and weight of the animals *(1,7,27–29)*. These apparent decreases in metal accumulation in relation to size could be a result of growth dilution *(1,30)*. An alternative explanation to lower metal concentrations in larger animals could be gonadal development. Larger clams have a higher probability of spawning. Some metals are essential elements that might actively be transferred and accumulated in the gonad *(31)*. When mature animals spawn, some of these metals might be lost as eggs are released into the water *(32)*. Additionally, spawning reduces gonad weight and affects the total weight of the organism. Hence, we would expect lower concentrations of essential metals in recently spawned animals and higher levels of metals that are not used during reproductive activities. For example, Cu is probably transferred to eggs because it forms a part of hemocyanin, the oxygen-binding molecule in mollusks *(33)*. A decline in Cu in the adult clam would be expected after the spawning period. May and June are the months with the greatest spawning activities for chocolate clams *(34)*. Otchere *(4)* found a negative correlation between Cu and the size of the bivalve *Anadara senilis*, but only during the dry season. We sampled in April, when a proportion of the clam population was probably spawning. If Cu is transferred to eggs, we can expect lower levels of Cu in the clams in April and higher in October, and in accordance, Cu levels were higher in October (10.1 ± 0.2 vs 6.7 ± 0.4 μg/g dry weight; $p < 0.05$). Additionally, a negative correlation between tissue weight and Cu occurred only in April (Table 4). These results were not universal. Lowest levels of Cu occurred in October at sites 1 to 4, but the highest levels at sites 5, 6, and 7. Only Zn at site 5 and Pb at site 4 were at significantly lower levels in October as compared to April, whereas Mn was higher in October at sites 1 and 5. This unclear effect could be a result of the large variance in tissue weight between April and October.

Based on the results obtained in this work, we recommend that consumption of chocolate clams be reduced before the rainy season because of the possibility of Pb and Cd accumulation to levels that, although below the limits recommended for daily ingestion, are still high and potentially hazardous to human health if clams are consumed regularly. Collecting clams at sites that are apparently contamination-free or far from anthropogenic disturbance is no guarantee that metals will be in low concentrations.

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