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Trace Metals in Two Geoduck Clams (*Panopea generosa* and *P. Globosa*) Exploited for the Regional Market from Two Areas of Northwest Mexico

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Abstract

Biological and fishery features of *Panopea* sp. clams have been studied in northwest Mexico because of their importance for human consumption. However, the content of pollutants in their tissues, along with their implications have not been addressed yet. The concentrations of cadmium (Cd), copper (Cu), mercury (Hg), and zinc (Zn) in soft tissues of *Panopea generosa* and *P. globosa* clams were examined in this region by atomic absorption spectroscopy. The metal concentrations (µg/g wet weight) ranged from 6.5 to 14.2, 0.97–8.09, 0.60–1.18, and 0.01–0.07, for Zn, Cu, Cd, and Hg, respectively. This study proposes that metal presence is related to weathering, upwelling, and drainage from adjacent agricultural lands to the coast. According to the Official Mexican Standard (NOM-242-SSA1-2009) and the World Health Organization (WHO, 2022), the metal content in siphon tissue indicates safe levels for human consumption.

Keywords Mercury · Cadmium · Copper · Zinc · Health Risk · Gulf of California

Introduction

Metals occur naturally in aquatic ecosystems and are leached from soil, rocks, and the atmosphere into natural waters. Generally, they do not result in any serious or deleterious effects on human health; however, anthropogenic activities promote an increase in environmental metal pollution. Northwest Mexico – particularly the Gulf of California (GC) ecoregion – hosts around 10 million inhabitants and supports economic activities, such as tourism, mining, agriculture, fishing, and shrimp farming, which pose a threat to its rich biodiversity, endemism, high biological productivity, and its environmental health. The main activities that produce trace-metal emissions in the GC ecoregion are gold mining and refining, Hg mining, thermoelectric plants, agriculture, aquaculture, and deforestation (Páez-Osuna et al. 2017).

Therefore, it is important to know metal bioavailability in organisms of ecological and commercial importance from these environments to establish their baseline and risks involved for human consumption of these organisms. *Panopea* sp. clams (family Hiatellidae) – considered as a delicacy food – are mud-burrowing mollusks of commercial importance for human consumption. In Mexican coasts, two species of this genus exist, the Pacific geoduck *P. generosa* (Gould 1850) and the Cortez geoduck *P. globosa* (Dall 1898). In Mexico, studies about *Panopea* clams have been focused on age, growth, mortality, cultures, genetics, ecology and fisheries (Cortez-Lucero et al. 2011; Suárez-Moo et al. 2013; Cubillo et al. 2018). Conversely, several studies have reported the potential use of bivalves as metal

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Fig. 1 Study area indicating the sites (black circles) from the coast of Baja California where wild *Panopea* spp. clams were collected

pollution bioindicators in marine ecosystems (Rainbow 2018; Lu et al. 2019).

Panopea clams are important to study considering that they fulfill most of the required features of ideal biomonitor organism (sedentary, filter-feeding, abundance, availability, and commercial importance) (Páez-Osuna and Osuna-Martínez 2011) of pollutants. In addition, *Panopea* clams exhibit a longevity higher than other clam species, thus, they could reflect a good integration of metal content over long periods of time. According to the open literatures, only two published studies are available on metal content in *Panopea abrupta* clams (Oliveira et al. 2011; Tong et al. 2016). Therefore, the aim of this study is to analyze Cd, Cu, Hg, and Zn in soft tissues (siphon, gills, mantle, and viscera) of *Panopea* clams from the west coast of Baja California (*P. generosa*) and the Upper Gulf of California (*P. globosa*), Mexico. These areas are regularly exploited to satisfy the demand for these clams in the regional market of Baja California, the USA, and surroundings. The hypothesis is that: (i) higher levels of Cu and Zn can be found in all the studied tissues and (ii) potentially toxic Hg and Cd can be found at lower levels in the edible tissue of both species. The siphon tissue was contrasted with levels of other commercially important clams and with the maximum permissible limits (MPL) of standards for safe human consumption.

Methods and Materials

A total of 30 specimens of *P. globosa* clams were collected from Puertecitos, San Felipe (upper Gulf of California), while five specimens of *P. generosa* clams were collected from Punta San Antonio, El Rosario (west coast Baja California Peninsula, Mexico) (Fig. 1) – all captured by autonomous diving (December 2015) – according to the Mexican regulation (NOM-014-SAG/PESC-2015). The clam shells were measured (mm), and total weight (shells and soft tissue, g) was recorded. The condition index (CI; Walne and Mann 1975) was calculated with the equation CI = soft tissue dry weight (g) /shell dry weight (g). The organisms were dissected and separated in siphon, mantle, viscera, and gill tissues, which were weighed, freeze-dried (72 h, -53 ºC, 0.124 mBar), and their moisture contents were recorded. Finally, tissue samples were stored in polyethylene containers and kept frozen (-20 ºC) until digestion. Glassware and polyethylene containers were previously acid cleaned (Bergés-Tiznado et al. 2015). Specimens were analyzed individually, aliquots of samples (0.260 ± 0.008 g of dry tissue) were pre-digested by duplicate at room temperature with 5 mL of HNO_3 (concentrated 70%; trace metal grade JT Baker). Complete digestion was carried out in Savillex Teflon bombs (120 °C for 3 h). Digested samples were diluted with Milli-Q water (volume 20 mL) and stored in polyethylene containers. Blanks and reference materials DOLT-4 (Dogfish liver; NRC 2008) and NIST-2976 (Mussel tissue) were digested (one in each batch per 25 samples) with the same procedure to evaluate accuracy and precision (Bergés-Tiznado et al. 2015). The analyses were carried out by atomic absorption spectrometry (AAS; Varian SpectraAA 220); Cu and Zn with flame detection, Cd with graphite furnace detection (Varian GTA110), and Hg with cold vapor detection. The recoveries of the certified materials were 88.6 ± 8.3% for Hg, 89.8 ± 9.0% for Zn, 96.4 ± 9.1% for Cu, and 104.2 ± 10.4% for Cd. All the metal concentrations were expressed as wet weight (ww) basis.

An exploratory analysis of the data was performed using R language (3.5.3) for normality tests (Kolmogorov-Smirnov, Lilliefors, and Shapiro-Wilk W) and homoscedasticity tests (Levene). Since there was a limited number of *P. generosa* specimens (five clams), the 'bootstrap' method (assuming 1000 observations) was employed (Efron and Tibshirani 1991); parametric analyses were used. The differences among metal concentrations and the studied tissues of each species were made by a One-way analysis of variance (ANOVA) and multiple comparisons by a Post Hoc Tukey's Honestly Significant Difference (HSD) test. A Student's T test was used to establish differences in the levels of metals between each clam species. Pearson Product-Moment correlations (r) were used to determine the association among

Table 1 Metal concentrations (mean ± standard error (SE), µg/g wet weight) in the siphon, gills, mantle and viscera of two clam species

Species Tissues	n	Hg	Cd	Cu	Zn
P. generosa	5	$0.013 \pm 0.002^{a,1}$	$0.38 \pm 0.19^{a,1}$	$2.61 \pm 2.81^{a,1}$	$12.28 \pm 5.02^{a,1}$
Siphon		$0.013 \pm 0.009^{a,1}$	$0.54 \pm 0.14^{a,1}$	$2.31 \pm 0.71^{a,1}$	$14.31 \pm 5.02^{a,1}$
Gills		$0.017 \pm 0.004^{a,1}$	$0.59 \pm 0.17^{a,1}$	$10.19 \pm 6.07^{a,b,1}$	$50.34 \pm 19.43^{b,1}$
Mantle		$0.019 \pm 0.004^{a,1}$	$0.97 \pm 0.19^{b,1}$	$10.84 \pm 6.10^{b,1}$	$39.16 \pm 11.77^{b,1}$
Viscera					
P. globosa	30	$0.014 \pm 0.005^{a,1}$	$0.63 \pm 0.21^{a,2}$	$0.70 \pm 0.67^{a.2}$	$5.55 \pm 1.05^{a,2}$
Siphon		$0.029 \pm 0.021^{b,1}$	$0.82 \pm 0.33^{a,1}$	$1.55 \pm 1.48^{a,1}$	$7.27 \pm 1.38^{a,2}$
Gills		$0.033 \pm 0.009^{b,2}$	$0.62 \pm 0.29^{a,1}$	$2.65 \pm 3.78^{a,2}$	$5.79 \pm 2.46^{a,2}$
Mantle		$0.082 \pm 0.030^{c,2}$	$1.21 \pm 0.45^{b,1}$	$7.64 \pm 11.42^{b,1}$	$10.10 \pm 4.17^{b,2}$
Viscera					

Different superscript letter indicates significant differences ($p < 0.05$) of mean concentrations among the tissues of each species; different superscript number indicates significant differences ($p < 0.05$) of mean concentrations between species; n, number.

the study variables; in all cases, the level of significance was $p < 0.05$ (Zar 1999). The non-cancer risk assessments were calculated as the individual target hazard quotient (THQ) and the hazard index (HI) by comparing and estimate of exposure to a reference dose (RfD) for oral exposures (EPA 2005): THQ = [EF x ED x FIR x C/RfD x BW x AT] x 10^{-3} and HI = ΣTHQ; where EF is an exposure frequency of 365 days/year; ED is a 70-year exposure period; FIR means the food ingestion rate of 0.44 g/day for bivalve consumption in Mexico (SEMARNAT 2020); C is the mean concentration of the element (mg/kg); BW is the population body weight of 75 kg for men and 65 kg for women; and AT is the average exposure of 25,500 days. Risk will exist if THQ or HI > 1. The RfD (mg/kg BW-day) for Hg (as methylmercury), Cd, Zn were taken from the IRIS Assessment Base (EPA, 2022); Cu has not been evaluated. Finally, a safe intake was calculated, according to the provisional tolerable intake per body weight (BW) set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA); the data for each element used were (WHO, 2022): Cd 25 µg kg^{-1} $BWmonth^{-1}$; Cu 0.5 mg kg^{-1} $BWday^{-1}$; Methyl-Hg 1.6 µg kg^{-1} $BWweek^{-1}$; Zn 0.3 mg kg^{-1} $BWday^{-1}$.

Results

Mean total weight (g) of the whole specimens, mean total length of the valves (mm), and the CI were statistically different ($p < 0.05$) between species. *Panopea globosa* measured the largest lengths (121–168 mm), whilst *P. generosa* showed the highest weights (550–880 g). Mean CI of *P. globosa* (1.3 ± 0.4) was lower than *P. generosa* (5.4 ± 0.9). The general trend of metal content in both clam species followed the order Zn > Cu > Cd > Hg. *Panopea generosa* accumulated higher concentrations ($p < 0.05$) of Zn compared to *P. globosa* in all tissues. Nonetheless, levels of Cu in the siphon and mantle of *P. generosa* were higher than *P. globosa*, and Cu levels in the viscera and gills were statistically the same for both species ($p > 0.05$, Table 1). Significantly negative correlations ($p < 0.05$) were found among the valve length of *P. globosa* with Zn in the mantle (r = -0.38) and viscera (r = -0.40), as well as Cu in the siphon (r = -0.38).

Concerning Cd and Hg, significant differences were found ($p < 0.05$) in the Hg bioaccumulation between both species with higher levels of Hg in gills, mantle, and viscera of *P. globosa* compared to *P. generosa*. According to the organ/tissue distribution, these clams show the trend of metal accumulation viscera ≥ mantle ≥ gills > siphon (Table 1).

Discussion

The order of metal distribution between tissues agrees with most literature on metal content in the tissues of different aquatic organisms (Frías-Espericueta et al. 2009) since the digestive gland (in viscera) in clams is the main organ for metal accumulation. These results are of particular importance since the siphon is the organ/tissue mainly consumed by humans. As it was expected, higher levels of Zn and Cu are related to the known role of essential metals, which provide some component of biochemical or enzymatic systems. The negative correlations between valve length and essential metals imply that, as the clams grow the levels of Zn and Cu decrease. This tendency has been reported previously for other bivalves, indicating that a metal-bivalve species-specific relationship exists. The observed decrease in older organisms could be due to a combination of growth dilution and the effect of the use of these metals in the reproductive cycle (Delgado-Alvarez et al. 2019).

Cadmium exhibited higher levels ($p < 0.05$) in the siphon and gills of *P. globosa* than *P. generosa*. Groslin (2004) explains that the gills, kidney, and digestive gland (these last two located in the viscera of *Panopea*) are considered as the most important bioaccumulation sites. Trace metals are commonly sequestered by metallothioneins in the gill,

Table 2 Metal contents (mean ± standard deviation (SD) or range) in the soft tissue (µg/g, dry weight) of clam species from Mexico and diverse sites of the world

Species	Hg	Cd	Cu	Zn	Site
Venerupis decussatus[1]	0.08–0.6	1.4–3.7	67.2-157.3	67.2-157.3	Coastal region, Morocco
Panopea abrupta[2]	-	0.4–0.6	0.9–9.2	29–265	Ketchikan, Alaska
Ruditapes philipinarum[3]	0.2–0.3	0.4–0.8	6.1–7.6	-	Ria Aveiro, Portugal
Mya arenaria[4]	-	1.6	12.4	80.0	Gdansk bay, Poland
Scrobicularia plana[5]	-	8.5	136	1883	Peel estuary, United Kingdom
Tridacna maxina[6]	-	1.07–1.86	2.26–3.06	14.39–14.49	Red Sea, Egypt
Macoma balthica[7]	-	9.4	224	1510	Severn estuary, United Kingdom
Amiantis umbonella[8]	0.5–1.2	0.007–0.013	2.1–3.2	2.0-6.6	Arabian Gulf, Saudi Arabia
Protapes sinuosa[8]	0.5–1.2	0.006–0.014	2.0-3.3	2.1–6.4	
Ruditapes philippinarum[9]	0.2-1.0	0.3-1.0	7.7–13.1	76–151	Venice lagoon, Italy
Solen marginatus[9]	0.15–2.25	0.3–1.2	10.5–18.1	50–90	
Ruditapes philippinarum[10]	-	0.35	3.02	51.4	Baseline level for China
Mexico					
Chione sp.[11]	-	0.8	13.4	44.0	Upper Gulf of California
Chione gnidia[12]	-	0.21–1.28	4.8–23.0	92.4–246.0	Guaymas, Sonora
Laevicardium elatum[12]					
Chione californiensis[13]	-	0.42 ± 0.14	5.70 ± 0.84	91.8 ± 7.8	Upper Gulf of California
Chione californiensis[14]	-	2.5	9.9	377	Altata-Ensenada del Pabellón lagoon
Chione subrugosa[14]	-	2.1	48.5	347	
Corbicula fluminea[15]	0.09 ± 0.01	0.45 ± 0.11	9.0 ± 0.64	65.9 ± 10.0	Coatzacoalcos estuary
Polymesoda caroliniana[15]	0.15 ± 0.04	1.05 ± 0.11	11.1 ± 2.8	126.7 ± 46.5	
Megapitaria squalida[16]	0.99 ± 0.81	4.5 ± 0.2	8.8 ± 1.3	68.9 ± 37.6	Navachiste lagoon, NW
Panopea generosa[17]	0.06–0.12*	2.0-4.8*	12.8–69.1*	60.9-264.9*	El Rosario, Baja California
Panopea globosa[17]	0.09–0.23*	4.2–9.1*	4.4–59.6*	36.1–74.7*	San Felipe, Baja California

[1]Maanan 2008; [2]Oliveira et al. 2011; [3]Costa et al. 2020; [4]Szefer et al. 1990; [5]Southgate et al. 1983; [6]Mohammed et al., 2013; [7]Bryan et al. 1980; [8]El-Sorogy et al. 2016; [9]Sfriso et al. 2018; [10]Lu et al. 2019; [11]García-Hernández et al. 2001; [12]Mendez et al., 2002; [13]Cadena-Cárdenas et al. 2009; [14]Páez-Osuna et al. 1993; [15]Ruelas-Inzunza et al. 2009a; 2009b; [16]Delgado-Alvarez et al. 2019; [17]this study; -, not analyzed; *average of the soft tissue

mantle, and digestive gland tissue or by lysosomes in the digestive gland and kidney cells (Rainbow 2018). These results (Cd, Cu, and Zn) were higher, compared with metal content in the edible muscle of *P. abrupta* collected in Alaska (Table 2; Oliveira et al. 2011), which may reflect the differences in natural processes (climate, weathering and upwelling events) and the pollution degree between both environments (subtropical and polar).

It is important to highlight that the mean Hg concentration of both clam species was lower, compared to other studied species (Table 2). The Cd content in *Panopea* species from this study was lower than other bivalves as oyster *C. gigas* and mussel *M. galloprovincialis* from the coastal region of Morocco (Maanan 2008), which could be related to the last study site, since it is the most urbanized and industrialized area. Similarly, Cd content in *P. generosa* and *P. globosa* were lower than mussel *M. californianus* (15.0 µg/g, dw) sampled previously from a nearby zone where *P. generosa* clams were collected (San Quintín, Mexico; Gutiérrez-Galindo et al. 2014). Such differences could have been related to the distinct species (metabolism) involved and the different exposure and sampled dates. Gutiérrez-Galindo et al. (2014) explained that high Cd levels have a natural origin associated to upwelling. However, these comparisons should be considered with caution, since oysters and mussels live in a habitat attached to mangrove roots or to rocks, which are exposed to water movement. In contrast, *Panopea* clams are the largest burrowing clams and spend all their lives inside the sediment. Moreover, they are one of the longest-living animals of any type with a typical lifespan of 140 years (Cortez-Lucero et al. 2011). Accordingly, the metabolic capacity to remove and/or excrete metals from their tissues is highlight, when compared with other bivalves. Conversely, Cd levels resulted higher than those reported for other clam species also from the Moroccan coasts (Maanan 2008), and from other sites inside the Gulf of California (Table 2).

Copper concentrations resulted higher than all the other clam species (Table 2), such as *Chione gnidia* and *L. elatum* from Mexican coasts (Méndez et al. 2002), *Chione* sp.

Table 3 Risk assessments Target Hazard Quotient (THQ) and Hazard Index (HI) from a consumption of 0.44 g/week for adults (men 75 kg BW and women 65 kg BW) of two clam species

Risk assessment	*P. generosa* THQ		*P. globosa* THQ	
	Men	**Women**	**Men**	**Women**
Hg	0.0008	0.0009	0.0008	0.0009
Cd	0.0023	0.0026	0.0037	0.0043
Zn	0.0002	0.0003	0.0001	0.0001
HI (ΣTHQ)	**0.0033**	**0.0038**	**0.0046**	**0.0054**

BW = body weight

from the upper Gulf of California (García-Hernández et al. 2001; Cadena-Cárdenas et al. 2009), C. *californiensis* from coastal lagoons of the Gulf of California (Páez-Osuna et al. 1993), and *Ruditappes philipinarum* from Ria Aveiro, Portugal (Costa et al. 2020). Inclusive, the Cu levels of geoduck are higher than those from Coatzacoalcos estuary where *C. fluminea* and *P. caroliniana* are exposed to contamination related to the oil industry (Ruelas-Inzunza et al. 2009a, b). Moreover, when these results are compared with mussels from a near zone (San Quintin, Mexico; Gutiérrez-Galindo et al. 2014), higher Cu levels were observed in both *Panopea* clams. As it was previously indicated, such differences could be related to the distinct species involved. Agricultural activity is an important factor that could result in high metal concentrations in the west coast of the Baja California Peninsula (specifically near San Quintin). Continued phosphate fertilization in agricultural soils could increase its metal content, and then be transferred to adjacent ecosystems such as coastal environments. The San Quintin Valley is one of the most important agricultural zones from NW Mexico and it is located North from El Rosario (collection site of *P. generosa*). Therefore, the transported material from adjacent agricultural lands to the coastal zone could occur and influence the metal content of clams. However, such Cu and Zn levels could be simply associated to the requirements of these two species.

When compare to the maximum permissible limits of national (NOM-242-SSA1-2009) and international (WHO, 2022) standards for safe human consumption, levels of non-essential metals (Hg, Cd) in siphon tissue resulted to be low. Besides, the risk assessment (THQ and HI) evaluated showed no adverse health risk from the siphon consumption. Evaluation of the data (Table 3) for all the elements in adult men and women showed a THQ < 1 as well as an HI < 1, for both *Panopea* species.

To conclude, safe daily intakes were evaluated for each element to avoid adverse health effect risks, considering an adult person (70 kg bw). The daily portions vary among elements and clam species, but they were considered high to be consumed by the general population. For example, to be in Hg risk, an adult must consume daily more than 1.6 kg ww of siphon of either species. Regarding Cu, the consumption must be over 50.0 and 13.4 kg ww of siphon of *P. globosa* and *P. generosa*, respectively, to be in danger. The consumption to be at Zn risk must be higher than 3.8 and 1.7 kg ww of siphon of *P. globosa* and *P. generosa*, respectively. The lowest safety daily consumption was for Cd with rations of 0.1 and 0.2 kg ww of siphon of *P. globosa* and *P. generosa*, respectively. Average metal levels were higher in the mantle than in the siphon for both clams; thus, the daily rations calculated for this tissue intake were lower. Mercury health effects might be evidenced if an adult consumes more than 0.5 and 0.8 kg ww of mantle every day of *P. globosa* and *P. generosa*. Cadmium consumption must be 0.1 kg ww of mantle of each species to avoid risk and Cu 13.2 and 3.4 kg ww of mantle for *P. globosa* and *P. generosa*, respectively. Finally, a daily safe consumption of mantle for Zn would be 3.6 and 0.4 kg ww for *P. globosa* and *P. generosa*, respectively. According to the results, daily rations are unreal to be consumed by people, and consequently, the intake would not represent any adverse health risks.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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