

TRACE METALS IN TWO WILD POPULATIONS OF THE SQUALID CALLISTA CLAM (*Megapitaria squalida*) IN THE SOUTHEASTERN GULF OF CALIFORNIA, MEXICO

Metales traza en dos poblaciones silvestres de la almeja callista escuálida (*Megapitaria squalida*)
en el sureste del Golfo de California, México

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ABSTRACT

The squalid callista clam (*Megapitaria squalida*) is a popular raw seafood item for human consumption; however, as a filter feeder, this clam accumulates heavy metals from natural and anthropogenic sources. The concentrations of copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), zinc (Zn), arsenic (As), and mercury (Hg) in sediments and the soft tissues of *M. squalida* from two sites on the southeastern coast of the Gulf of California, Mexico, were evaluated from April 2016 to April 2017 on a monthly basis. The metal concentrations in sediments from both sites did not exceed the Mexican and international regulations. Concentrations of Cd and Pb in the clams from Altata bay (2.49 and 5.68 µg/g dw, respectively) and Agiabampo bay (2.38 and 5.54 µg/g dw, respectively) exceeded the permissible limits recommended by Mexican sanitary regulations, thus representing a threat to human health. The higher values of Cd, As, and Hg obtained for the biota sediment accumulation factor in both sampling areas indicate that squalid callista is a strong accumulator of these metals. The metal burdens in sediments and *M. squalida* soft tissues are influenced by chemicals from agriculture and aquaculture, as well as urban sewage disposal near both sites. This study brings useful information on metal bioaccumulation in one of the most important commercial clam species on the Pacific coast.

Palabras clave: toxicología, almeja chocolata, bioacumulación de metales, sedimentos, Sinaloa

RESUMEN

La almeja callista escuálida (*Megapitaria squalida*) es un marisco popular que se consume crudo, pero por ser filtrador, puede acumular metales pesados de fuentes naturales y antrópicas. En el presente trabajo se evaluaron, de abril de 2016 a abril de 2017, las concentraciones mensuales de cobre (Cu), cadmio (Cd), cromo (Cr), níquel (Ni), plomo (Pb), zinc (Zn), arsénico (As) y mercurio (Hg) en sedimentos y tejidos blandos de *M. squalida* de dos sitios de la costa sureste del Golfo de California, México. Los niveles de metales en los sedimentos de ambos sitios no excedieron las regulaciones mexicanas e internacionales. Las concentraciones de Cd y Pb en las almejas de la bahía de Altata (2.49 y 5.68 µg/g ps, respectivamente) y la bahía de Agiabampo (2.38 y 5.54 µg/g ps, respectivamente) sí excedieron los límites permisibles recomendados por las regulaciones sanitarias mexicanas, lo que representa una amenaza para la salud humana. El factor de acumulación de sedimentos en la biota para las dos áreas muestreadas indica que la almeja callista escuálida es un acumulador fuerte de Cd, As y Hg. Las concentraciones metálicas en los sedimentos y los tejidos blandos de *M. squalida* están influenciadas por los productos químicos utilizados en la agricultura y la acuicultura, así como por el suministro de aguas residuales urbanas cercanas a ambos sitios. Este estudio brinda información útil sobre la bioacumulación de metales en una de las especies de almejas comerciales más importantes de la costa del Pacífico.

INTRODUCTION

Trace metals reach coastal regions through continental runoff and atmospheric transport by natural and anthropogenic causes (Abdel-Ghani 2015). Nevertheless, they can accumulate at high concentrations in both the aquatic environment and marine organisms due to sewage disposal from human activities such as mining, livestock, poultry, agriculture, and aquaculture. Hazardous pollutants in wastes from anthropogenic activities are discharged into local irrigation systems and from there to estuaries and coastal ecosystems (Rainbow 2002).

Occupying the second trophic level of aquatic ecosystems, sessile filter-feeding bivalve mollusks inevitably accumulate high levels of metals in their soft tissues, representing a danger for human consumption. Marine bivalves feed selectively, filtering large volumes of seawater through their gills (Silvester et al. 2005) with which they trap suspended materials and sediments, phytoplankton, fecal pellets, detritus, and high molecular weight substances (Liu and Deng 2007) that are eventually ingested. Thus, metals associated with the aqueous phase, food, and sediments accumulate in the shells (Cravo et al. 2002) and soft tissues of these organisms (Góngora-Gómez et al. 2018).

Due to their sensitivity and rapid response to pollutants, bivalves such as mussels and oysters (Bray et al. 2015) are used as biomonitors of metal contamination in aquatic systems (Kanthai et al. 2014), the bioavailability of contaminants, and the degree of pollution. To a lesser extent, clams also serve as

sentinel organisms for marine eco-toxicological tests to ensure coastal water quality with respect to metals. For instance, Bendell (2009) performed cadmium analysis on oysters, mussels, clams, and scallops sampled from commercial and natural sources on the west coast of British Columbia, Canada, and Kehrig et al. (2006) reported the levels of methyl and total mercury in mussels, oysters, and clams from two estuaries in Rio de Janeiro, Brazil. In both studies, mussels and oysters showed higher metal accumulation than clams and scallops. The latter two are part of the sedentary non-sessile benthic fauna for which metal accumulation levels strongly depend on the metals associated with particulates that settle in bottom sediments (Inengite et al. 2010) and on the specific bioavailability of different trace metals (Ferreira et al. 2004).

Megapitaria squalida, commonly known as the squalid callista clam, represents one of the most important bivalve species on the southeastern coast of the Gulf of California, Mexico (Arellano-Martínez et al. 2006). This clam, which is naturally distributed from Baja California to Peru, digs in sandy clay sediments in the intertidal zone to depths of approximately 160 m (Coan and Valentich-Scott 2012). It is a popular raw seafood for human consumption with wild banks located in coastal lagoon systems in the southeastern Gulf of California, where intense anthropogenic activities also occur. Thus, these areas are under permanent pressure and the trace metal burdens in *M. squalida*, consequently, represent a potential risk for human health.

Due to the significance of copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), arsenic (As), zinc (Zn), and mercury (Hg) to public health (WHO 1996), their high degree of toxicity (ATSDR 2006), and their routine association with human activities around the sample sites (Gómez-Arroyo et al. 2013), the levels of these metals were assessed both in sediments and in the soft tissues of *M. squalida* from two locations on the southeastern coast of the Gulf of California during 13 consecutive months. The levels were evaluated based on national and international permissible levels for human consumption.

MATERIALS AND METHODS

Study area

Two sites along the north central coast of Sinaloa, Mexico, with different sources of pollution were selected for this study. Site 1 was located in Altata Bay (24° 20'–24° 35' N, 107° 20'–107° 55' W) in the Navolato municipality, which is a marine environment with an outlet providing a permanent connection to the Gulf of California. The main tributary of Altata Bay is the Culiacan valley, an extensive agricultural area where the primary economic activities are centered on El Dorado, Rosales, and La Primavera sugar mills, from which wastes are routed to irrigation district No. 10, and subsequently discharged directly into the bay (Gaxiola 2003). The main anthropogenic activities in this region are agriculture, fishing, urbanization, and tourism (Frias-Espicueta et al. 2014). Site 2 is located in Agiabampo bay (26° 06'–26° 32' N, 109° 01'–109° 20' W) in the Ahome municipality, between the El Fuerte and Mayo rivers with a permanently open mouth to the Gulf of California which allows the seawater flux in the bay and is surrounded by sand bars. This area is used for agriculture (Carrizo and Fuerte-Mayo drainages) and aquaculture (Colín-Rangel 2007).

Trace metal analysis

From April 2016 to April 2017, approximately 500 g of sediment were collected monthly from each site following standard USEPA (2001) procedures, kept in acid-cleaned plastic bottles, and transported to the Laboratorio de Malacología of the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Sinaloa, Instituto Politécnico Nacional (IPN-CIIDIR-Sinaloa), where they were frozen and stored. The frozen samples were freeze dried at low temperature and high vacuum (–53 °C and 133×10^{-3} mBar, respectively) for three

days to remove the moisture, then passed through a 2 mm sieve and 1 g of the sample was placed in acid-cleaned glass bottles prior to digestion.

To limit the effect of size as a source of variation, a total of 24 clams of approximately equal length (55.76 ± 5.09 and 61.58 ± 5.19 mm, for Altata and Agiabampo, respectively) were hand collected monthly from each site, thoroughly rinsed with seawater to remove adhered algae and sediments, stored on ice in clear polyethylene bags, and transported to IPN-CIIDIR-Sinaloa, following SSA (1994) methods for cleaning, sacrificing, and shucking. The soft tissues were removed from the clam shells using a stainless-steel knife and thoroughly washed with double distilled water. Samples were subsequently freeze dried (–53 °C and 133×10^{-3} mBar), pulverized, and homogenized by quartering; thus, all fractions of the sample were equal in composition. Dry weights (dw) of the samples were obtained by using a digital analytical balance (EA Adam, 0.001 g). All chemicals used in this study were GR grade (Merk Company). All materials were first cleaned with nitric acid (10 %) for a 24 h period then rinsed with double distilled water.

To analyze the metal burdens, the clam soft tissue samples (1–1.5 g dw) were digested with 3 ml of 70 % HNO_3 and 0.5 ml of 30 % H_2O_2 using a microwave digester (Parr Physica Multiwave Six Place) at 300 W for 5 min, then at 600 W for 10 min, which proved to be satisfactory for an adequate digestion of samples. Digestions were performed and stored until metal analysis as described by Góngora-Gómez et al. (2018).

All metals were analyzed at the Laboratorio de Análisis y Monitoreo Ambiental of the Centro Interdisciplinario de Investigaciones y Estudios sobre el Medio Ambiente y Desarrollo (IPN-CIEMAD); the concentrations of As and Hg were analyzed by atomic absorption spectroscopy (AAS) with cold vapor generation (Perkin-Elmer, Analyst 100, coupled MHS 15), and the rest of metals (Cu, Cd, Cr, Ni, Pb, and Zn) by using flame AAS (Perkin-Elmer, PinAAcle 900T). For quality assurance, the standard reference material (1566b for oysters, available at the National Bureau of Standards, NBS), reagent blanks, and duplicate samples were run with each digest. Mean recovery values were Cu = 93.03 %, Cd = 99.11 %, Cr = 91.25 %, Ni = 96.34 %, Pb = 97.72 %, Zn = 99.78 %, As = 95.30 % and Hg = 97.29 %. Heavy metal concentrations in sediment and soft tissue of clams were reported as dry weight ($\mu\text{g/g}$). The detection limits for Cu, Cd, Cr, Ni, Pb, Zn, As and Hg were 0.032, 0.008, 0.032, 0.046, 0.100, 0.0002, 0.039 and 0.0003 $\mu\text{g/g}$ dw, respectively.

The biota sediment accumulation factor (BSAF) indicates the efficiency of metal bioaccumulation in clam soft tissues regarding the concentration of metal in sediment; it was calculated for each site following Thomann et al. (1995):

$$\text{BSAF} = \frac{\text{concentration of metal in clam}}{\text{concentration of metal in sediment}}$$

Statistical analyses

All data were tested for normality, and statistical tests were selected accordingly. Descriptive statistics (mean, standard deviation, maximum, and minimum) were used to evaluate metal concentrations for each month and site. The coefficients of variation (CV) for the metal burdens were used to test the reliability of data. Student t-test was used to compare metal concentrations between sediments and clams from each site, and between sediments from both sites and clams from both sites. Spearman's rank order correlations were computed for the heavy metal levels in the sediments and clams from each site. Statistical analyses were performed ($p < 0.05$) using the STATISTICA (StatSoft, Tulsa, OK, USA) software package.

RESULTS

Metal levels in the sediments and clams from Altata and Agiabampo varied spatially (**Table I**). Zn had the highest monthly and mean values in both sediments (46.55 and $31.07 \mu\text{g/g dw}$ for Altata and Agiabampo, respectively) and clams (51.39 and $46.81 \mu\text{g/g dw}$ for Altata and Agiabampo, respectively). The CV values for metals fluctuated from 9.67% for Cu in sediments to 212.50% for Cr in clams at Altata Bay

TABLE I. METAL CONCENTRATIONS (MEAN \pm STANDARD DEVIATION, $\mu\text{g/g dw}$) IN SEDIMENT AND CLAMS FROM ALTATA AND AGIABAMPO BAYS, SINALOA, MEXICO

	Altata bay		Agiabampo bay	
	Sediment	Clam	Sediment	Clam
Cu	4.34 ± 4.42	6.22 ± 1.24	3.41 ± 0.78	5.56 ± 1.27
Cr	6.25 ± 1.25	0.08 ± 0.17	5.66 ± 1.85	0.20 ± 0.21
Cd	0.15 ± 0.14	2.48 ± 0.37	0.06 ± 0.12	2.38 ± 0.38
Ni	6.16 ± 1.09	3.50 ± 0.54	4.25 ± 1.71	2.47 ± 0.84
Pb	9.82 ± 2.36	5.68 ± 1.80	9.06 ± 1.93	5.54 ± 1.58
As	0.88 ± 0.23	4.47 ± 0.37	0.99 ± 0.39	4.52 ± 1.36
Zn	46.55 ± 9.56	51.39 ± 2.34	31.14 ± 9.04	46.81 ± 3.05
Hg	0.06 ± 0.02	0.28 ± 0.08	0.07 ± 0.03	0.32 ± 0.10

and from 6.51% for Zn in clams to 200% for Cd in sediments at Agiabampo Bay.

With the exception of Cd and Hg in sediments, the ranking of metal concentrations presented the same order that in sediments ($\text{Zn} > \text{Pb} > \text{Cr} > \text{Ni} > \text{Cu} > \text{As} > \text{Cd} > \text{Hg}$ at Altata and $\text{Zn} > \text{Pb} > \text{Cr} > \text{Ni} > \text{Cu} > \text{As} > \text{Hg} > \text{Cd}$ at Agiabampo) and clams ($\text{Zn} > \text{Cu} > \text{Pb} > \text{As} > \text{Ni} > \text{Cd} > \text{Hg} > \text{Cr}$ at Altata and $\text{Zn} > \text{Cu} > \text{Pb} > \text{As} > \text{Ni} > \text{Cd} > \text{Hg} > \text{Cr}$ at Agiabampo). Only the Zn levels in sediments and clams from Altata were similar ($p = 0.08$, **Table II**).

TABLE II. COMPARISON OF METAL CONCENTRATIONS BETWEEN SEDIMENTS AND CLAMS IN EACH SITE

	Altata bay		Agiabampo bay	
	t	p	t	p
Cu	-5.14	0.00*	3.36	0.00*
Cr	17.59	0.00*	10.36	0.00*
Cd	-20.33	0.00*	-19.04	0.00*
Ni	4.69	0.00*	3.97	0.00*
Pb	3.96	0.00*	4.98	0.00*
As	-29.64	0.00*	-8.82	0.00*
Zn	-1.77	0.08	-5.62	0.00*
Hg	-9.45	0.00*	-8.08	0.00*

t: Student t-test, p: level of significance

*Significant differences, $p \leq 0.05$

The concentrations of Cu, Ni, and Zn were significantly different ($p \leq 0.05$) between sediments from both sites and between clams from the two bays (**Table III**).

TABLE III. COMPARISON OF METAL CONCENTRATIONS IN SEDIMENTS AND CLAMS BETWEEN THE TWO SITES

	Sediment		Squalid callista	
	t	p	t	p
Cu	32.83	0.00*	17.40	0.00*
Cr	1.06	0.30	-1.26	0.23
Cd	1.96	0.07	0.88	0.39
Ni	3.62	0.00*	3.34	0.00*
Pb	1.41	0.18	0.20	0.83
As	-0.81	0.43	0.11	0.90
Zn	5.32	0.00*	4.22	0.00*
Hg	-0.88	0.39	-0.88	0.39

t: Student t-test, p: level of significance

*Significant differences, $p \leq 0.05$

The metal rankings for sediments and clams from both sample sites indicate that Zn was the most abundant element with a concentration ranging from 31.07 µg/g dw for Altata sediments to 51.39 µg/g dw in Altata clams.

None of the metals studied surpassed the permissible limits in sediments (CCME 2003; SEMAR-NAT 2007) from both sites; however, some metal contents in clams did exceed permissible limits. Cd and Pb contents in clams from Altata (2.49 and 5.68 µg/g dw, respectively) and Agiabampo (2.38 and 5.54 µg/g dw, respectively) were higher than permitted by Mexican regulations (2 and 1 µg/g dw, respectively) (SSA 1995, 2011).

At Altata, Cd and Pb were significantly correlated ($r = 0.55$) in clams and correlations were found between Ni and Cr, Pb and Cr, Pb and Ni, Zn and Cr, and Zn and Pb ($r = 0.63$ - 0.93) in sediments. For Agiabampo, significant correlations were obtained for Cd/Cr ($r = 0.73$), Pb/Ni ($r = 0.76$), and Zn/Cd ($r = 0.72$) in clams, and for Ni/Cr ($r = 0.65$), Ni/Cd ($r = 0.65$), and Pb/Cd ($r = 0.67$) in sediments.

Overall, the BSAF for Altata and Agiabampo presented the following order: Cd > As > Hg > Cu > Zn > Pb > Ni > Cr and Cd > As > Hg > Cu > Zn > Ni > Pb > Cr, respectively (**Table IV**). The clam soft tissues exhibited the highest levels of absorption capacity for Cd (10.75 and 7.07), As (5.42 and 5.25), Hg (4.83 and 5.04), Cu (1.44 and 1.74), and Zn (1.15 and 1.62) for Altata and Agiabampo, respectively.

TABLE IV. BIOTA SEDIMENT ACCUMULATION FACTOR (BSAF) OF *Megapitaria squalida* FROM ALTATA AND AGIABAMPO BAYS, SINALOA, MEXICO

	Altata	Agiabampo
Cu	1.44 ± 0.29	1.74 ± 0.70
Cr	0.01 ± 0.03	0.04 ± 0.04
Cd	10.76 ± 12.37	7.08 ± 15.16
Ni	0.59 ± 0.14	0.64 ± 0.26
Pb	0.63 ± 0.27	0.64 ± 0.21
As	5.42 ± 1.52	5.25 ± 2.39
Zn	1.15 ± 0.26	1.63 ± 0.49
Hg	4.83 ± 1.21	5.04 ± 1.94

DISCUSSION

Due to the ecological and commercial importance, as well as the high consumption demand for different mollusk species in the study region, several biomonitoring studies focusing on heavy metal accumulation

in a variety of bivalve species have been conducted and provide comparative data. Wild and cultivated oysters, pen shells, and clams have been used as biological monitors for heavy metals. For instance, Frías-Espéricueta et al. (2008) obtained higher Cu (21-73.2 µg/g dw) and Cd (1.8-7.2 µg/g dw) levels, but lower Pb (2.6-8.7 µg/g dw) burdens in a wild bank of the pleasure oyster (*Crassostrea corteziensis*) from the Altata bay. The Zn (1-1.66 µg/g dw), Cr (0.4-1 µg/g dw), Ni (0.33-2.3 µg/g dw), Pb (0.66-2 µg/g dw), and As (0.2-0.57 µg/g dw) concentrations reported by Góngora-Gómez et al. (2018) in the muscle of the pen shell (*Atrina maura*) cultivated at a farm located between the two sample sites were lower than the levels we obtained for Zn (2.44-5.16 µg/g dw), Cr (3.51-9.76 µg/g dw), Ni (2.49-8.55 µg/g dw), Pb (6.68-14.45 µg/g dw), and As (0.4-1.82 µg/g dw). Muñoz-Sevilla et al. (2017) registered variations of Cu (10.2-26.6 µg/g dw), Cd (0.8-3.8 µg/g dw), Pb (1.3-3.6 µg/g dw), Zn (57.2-219.4 µg/g dw), and Hg (0.02-0.11 µg/g dw) in Pacific oysters (*C. gigas*) cultivated near the Agiabampo bay. These variations can be attributed to differences in human activities around the study areas, latitude, environmental conditions, and the bivalve species studied.

Different baseline metal orders in sediments have been reported by Widmeyer and Bendell-Young (2008) in British Columbia, Canada (Cu > Zn, Pb > Cd), Tarique et al. (2012) (Pb > Cu > Cd > Hg) in the Kuwait bay, and Jonathan et al. (2017) (Zn > Cr > Ni > Cu > Pb > As > Cd > Hg) in the southwestern Gulf of California. In the case of Altata and Agiabampo, metal concentrations in sediments were affected by a number of factors, including sewage effluents from industry, urban sewage disposal, agricultural activities, and aquaculture farms close to the sample sites (Páez-Osuna et al. 2003, Hernández-Cornejo et al. 2005, Muñoz-Sevilla et al. 2017); also, by the relationship between grain size and texture and metal adsorption that in this case favors degradation of organic matter by tidal mixing (Forrest and Creese 2006), as well as the natural erosion of the rocks, dissolution processes, and water runoff from upstream of the estuaries (Murray and Busty 2015). The external and natural inputs at each specific locality may help to understand the differences among the aforementioned results.

The metal bioaccumulation orders observed in *M. squalida* from both sites differed from those reported by Tarique et al. (2012) (Zn > Cu > Cr > Ni > Hg > Cd > Pb) and Mohammad et al. (2017) (Zn > Pb > Cu > Cd) for the clams *Amiantis umbonella* in the Kuwait bay and *Donax semistriatus* in the Mediterranean Sea, respectively. Góngora-Gómez et al.

(2017) analyzed the metal concentrations in oysters (*C. gigas*) cultivated in a lagoon system between Altata and Agiabampo, registering the following rank order of accumulation: Zn > Cu > Cr > Cd > Ni > Pb > As > Hg, which is similar to those obtained by Osuna-Martínez et al. (2011) (Zn > Cu > Cd > Pb) and Vázquez-Boucard et al. (2014) (Zn > Cd > Pb) for the same oyster species in the studied region.

Several biotic factors affect metal bioaccumulation in bivalve mollusks. Some trace metals like Zn, Na, and Cu are essential for tissue formation, metabolic physiology, cellular metabolism, and nutrient synthesis and metabolism (Barile 2008), but levels exceeding normal requirements can impair growth, reproduction, and general development. Jara-Marini et al. (2013) stated that Cu levels in adult oysters decreased during the post-spawning event, whilst Lango-Reynoso et al. (2010) registered variations in Cd accumulation during different reproductive stages. Pérez-Osuna et al. (1995) concluded that concentrations of some metals vary seasonally with reproductive events and gonadal development after finding higher levels of Cu and Zn at the end of the reproductive cycle in oysters. These findings do not coincide with the results obtained in this study, since *M. squalida* from both sites did not show a normal seasonal pattern for bioaccumulation of the metals examined. On the other hand, no tools for evaluating clam reproductive phases were employed in this study, as this was not the goal of our research. In fact, no reports on the relationship between metal accumulation and reproduction of the squalid callista are available so far.

As with other bivalves, *M. squalida* is a popular raw dietary item not only in coastal areas of the Gulf of California, but also in cities where there is a high demand for this resource (Amezcuca-Castro et al. 2015). However, only a few studies have been conducted on heavy metals in the squalid callista along the coasts of the Gulf of California. Méndez et al. (2006) analyzed Pb, Ni, Cd, Mn, Zn, Cu, and Fe levels in *M. squalida* from an apparently contamination-free site in the southwestern Gulf of California, and concluded that this clam should be consumed before the rainy season due to Pb and Cd accumulation. Cantú-Medellín et al. (2009) stated that some antioxidant defense mechanisms in *M. squalida* changed in response to the bioaccumulation degree of some metals, such as Cd, Pb, and Fe. Through morphometry and condition index, Yee-Duarte et al. (2017) concluded that health of the squalid callista clam in Santa Rosalía, Baja California Sur, evidenced negative physiological effects, possibly caused by

contamination from metals produced by the local mining activity. Romo-Piñera et al. (2018) evaluated the total Hg concentration in the squalid callista, finding relatively low values of Hg (0.06-0.09 µg/g dw) in soft tissues that nonetheless represent a potential risk for human health.

These works were carried out in wild squalid callista populations from the southwestern Gulf of California. Only Frías-Espéricueta et al. (2008) recorded metal contents (Cd, Cu, Pb, and Zn) in oysters, mussels, and clams from the Altata-Pabellones lagoon system (southeastern Gulf of California) and found differences in metal bioaccumulation during the rainy and dry seasons, and between the different bivalve species. They concluded that the inner mangrove swamp species (*C. corteziensis* and *Mytella strigata*) bioaccumulated higher levels of Cd and Cu than *M. squalida*, which inhabits areas under marine influence. This confirms the strong influence of habitat on metal bioaccumulation in different bivalve species, as well as the need for more research related to heavy metal accumulation in *M. squalida* from natural banks at the southeastern coast of the Gulf of California.

Since limits for Zn, Cu, Cr, and Ni in soft tissue of clams have not yet been set in Mexico, the concentrations of these metals were compared with standards established in other countries. Zn (51.39 µg/g dw) in *M. squalida* from Altata and Hg (0.32 µg/g dw) in clams from Agiabampo surpassed the permissible limits established by the Ministry of Agriculture, Fisheries and Food (50 and 0.3 µg/g dw, respectively) in the UK (Sally et al. 1996). Cu, Cd, and Zn burdens were lower than those reported by Frías-Espéricueta et al. (2008) for *M. squalida* from Altata. However, Ni was lower than the level obtained by Méndez et al. (2006) in a wild population of squalid callista clam on the southwestern coast of the Gulf of California. Recently, Romo-Piñera et al. (2018) reported Hg at 0.05-0.09 µg/g dw in soft tissues of *M. squalida* from La Paz bay, Baja California Sur, Mexico, which is lower than the Hg levels in both bays examined in the present study. The presence of high levels of Cd and Pb in clams from both places is associated to phosphate fertilizers used in agriculture (Jara-Marini et al. 2013) and aquaculture and industrial influxes from the region (Luoma and Rainbow 2005), respectively.

A strong association has been reported between particular groups of metals, suggesting a possible common source (García-Rico et al. 2001, Jonathan et al. 2017, Muñoz-Sevilla et al. 2017). It is reported that in approximately 200 000 ha that surround the

two studied regions, the agriculture of different grains and vegetables, such as corn, tomato, and beans, is practiced; thus, enormous amounts of agrochemicals containing Cd, Ni, and Zn are used in fertilizers (Páez-Osuna et al. 1993), insecticides, fungicides, and herbicides (Gómez-Arroyo et al. 2013). These heavy metals are eventually leached from the soil and transported to the coastal lagoon systems inhabited by these clams (Páez-Osuna and Osuna-Martínez 2015). This was confirmed by the correlations found in the sediments between Ni/Cr, Pb/Cr, Pb/Ni, Zn/Cr, and Zn/Pb at Altata, between Ni/Cr, Ni/Cd, and Pb/Cd at Agiabampo, in the clam soft tissue between Cd/Pb at Altata, and between Cd/Cr, Pb/Ni, and Zn/Cd at Agiabampo.

Since both sites are connected to irrigation districts, they receive agrochemicals not only from agriculture and livestock industries, but also from shrimp aquaculture ponds (Hernández-Cornejo et al. 2005), resulting in high contamination influxes to the estuaries in the study area. There are reports for decades ago that the lagoon systems near our sample sites is receiving tons of inorganic nitrogen, inorganic phosphorous, fungicides, pesticides, and herbicides from anthropogenic activities highlighting that their influxes would increase over time (Escobedo-Urías 2010, Gómez-Arroyo et al. 2013). The presence of Pb and Cr relationships in sediments and clams reflects the impact of untreated sewage discharges from the different (domestic, aquaculture, and agriculture) activities (Wong et al. 2007) and industrial complexes (sugar mills) in the region (Gaxiola 2003).

The BSAF orders in our study reflect the high absorption capacity of Cd, As, and Hg; the moderate absorption capacity of Cu and Zn, and the low absorption capacity of Pb, Ni, and Cr in the soft tissues of clams. Soto-Jiménez et al. (2001) mentioned that the degree of metal availability from sediments is partially due to the exchangeable carbonates and organic phases made available to the organisms by water currents. Cd had the highest biosediment accumulation factor values at both sites, but the Cu and Zn concentrations in sediments were lower, suggesting a higher rate of accumulation of these metals by *M. squalida*. Pb, Ni, and Cr were generally lower in the clam soft tissues than in sediments from both sites, suggesting that the clams' capacity to regulate these metals was not surpassed by the metal levels in sediment, as observed by Mohammad et al. (2017) in the clam *D. semistriatus*. For the rest of the metals evaluated, factors such as metal concentration in water and clam metabolism (Páez-Osuna et al.

1995), among other internal and external factors, may partially explain the BSAF reported here.

CONCLUSIONS

All metals assessed were found in sediment and *M. squalida* soft tissue from both sites, confirming the strong influence from the various anthropogenic activities present around both studied bays. Domestic sludges and sewage waste from industry, agriculture, and aquaculture practices impacted the metal concentration patterns in sediments and *M. squalida*. Similar to the conclusions reported by Góngora-Gómez et al. (2018) for the pen shell *A. maura*, Cd and Pb burdens in the squalid callista clam exceeded the permissible limits for human consumption established by Mexican standards, posing a human health risk. Therefore, a continuous monitoring system is highly recommended and obligatory for the healthy production and consumption of this shellfish species.

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