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Heavy-metal contents in oysters (*Crassostrea gigas*) cultivated on the southeastern coast of the Gulf of California, Mexico

Contenido de metales pesados en ostiones (*Crassostrea gigas*) cultivados en la costa sureste del Golfo de California, México

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ABSTRACT

Background. For its flesh and flavor, the Pacific oyster *Crassostrea gigas* is cultivated worldwide, but as filter feeders, this bivalve bioaccumulates heavy metals from different pollution sources, rendering them unsafe for human consumption. **Goals.** We carried out this study to assess the heavy metal concentrations in cultivated oysters from a farm located on the southeastern coast of the Gulf of California during 2011. **Methods.** Oyster samples were analyzed monthly (March-December 2011) for cooper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni), lead (Pb), zinc (Zn), arsenic (As), and mercury (Hg). **Results.** The mean values (μg g^{-1}, dry weight) for each metal were Cu = 51.42 ± 25.92, Cr = 24.97 ± 32.38, Cd = 13.84 ± 4.22, Ni = 10.26 ± 12.18, Pb = 2.18 ± 1.28, As = 0.37 ± 0.08, Zn = 267.42 ± 92.29, and Hg = 0.02 ± 0.01. **Conclusions.** The results suggest that metal burdens could be influenced by anthropogenic activities such as agriculture and aquaculture surrounding the culture zone. Cu, Cr, Cd, and Pb levels (μg g^{-1}, fresh weight) were above the maximum permissible values and thus pose a threat to human health. Metal concentrations must be monitored periodically.

Key words: Aquaculture, bioaccumulation, Japanese oyster, metal toxicity, pollution.

RESUMEN

Antecedentes. El ostión del pacífico, *Crassostrea gigas,* es cultivado en el mundo por su sabor y textura de la carne, pero por ser un organismo que se alimenta mediante filtración, puede acumular metales pesados haciéndolo peligroso para su consumo. **Objetivos.** El presente estudio evaluó la concentración de metales pesados en ostiones cultivados en una granja localizada en la costa sureste del Golfo de California durante 2011. **Métodos.** Se tomaron muestras de ostión cada mes (marzo-diciembre 2011) para analizar el contenido de cobre (Cu), cadmio (Cd), cromo (Cr), níquel (Ni), plomo (Pb), zinc (Zn), arsénico (As) y mercurio (Hg). **Resultados.** Los valores promedio (μg g^{-1}, peso seco) de cada metal fueron: Cu = 51.42 ± 25.92, Cr = 24.97 ± 32.38, Cd = 13.84 ± 4.22, Ni = 10.26 ± 12.18, Pb = 2.18 ± 1.57, As = 0.37 ± 0.26, Zn = 267.42 ± 92.29 and Hg = 0.02 ± 0.01. **Conclusiones.** Los resultados obtenidos sugieren que la carga de metales pudo haber sido influenciada por actividades antropogénicas desarrolladas en los alrededores del área de cultivo, como agricultura y acuacultura. Los niveles de Cu, Cr, Cd y Pb en peso húmedo rebasaron los límites máximos permitidos representando un riesgo para la salud humana. La carga de los metales estudiados debe ser monitoreada periódicamente.

Palabras claves: Acuacultura, acumulación, contaminación, ostión japonés, toxicidad por metales.

INTRODUCTION

Heavy metals leached from soil and rocks to aquatic systems are naturally ubiquitous and pose minimal threats to the environment and human health (Páez-Osuna & Osuna-Martínez, 2015). Several anthropogenic sources, however, such as agriculture, aquaculture, and mining increase metal burdens exceeding natural levels. Since bivalves accumulate and exhibit efficient strategies to deal with the potential toxic effect of metals (Arifin & Bendell-Young, 1997), they are used as biomarkers for monitoring metal contamination in aquatic systems (Kanthai *et al.*, 2014).

There are reports from around the world that have used wild and cultured oyster populations from the genus *Crassostrea* spp. as biomonitors of trace metals. For instance, the spatial pattern of metal accumulation within intertidal bivalves was studied in England to measure the ecological impact of introducing *Crassostrea gigas* (Thunberg, 1873) in nature mussels (Bray *et al.*, 2015). Sarong *et al.* (2015) found high levels of Pb, Cd, and Zn in the body tissue of *C. gigas* harvested from the estuary of Lamnyong River, Indonesia, and Ochoa *et al.* (2013) concluded that metallic pollutants did not affect oyster cultures in Ebro Delta, Spain. Concentrations of heavy metals (Cu, As, Ni, Pb, and Cd) below the maximum levels for foodstuffs in Brazilian legislation were found in the mussel *Perna perna* Linnaeus, 1758 and *C. gigas* in production areas.

Specifically along the central eastern Gulf of California coast, Mexico, Bergés-Tiznado *et al.* (2013) evaluated the presence of arsenic compounds in the mangrove oyster *Crassostrea corteziensis* (Hertlein, 1951). Furthermore, Jara-Marini *et al.* (2013) compared the bioaccumulation of trace metals in six bivalve species. Analyzing soft tissue of *C. gigas* from culture sites along the east coast of the Gulf of California, Vázquez-Boucard *et al.* (2014) found Zn, Cd, and Pb concentrations above Mexican tolerance levels due to the presence of pesticides.

The Pacific oyster *Crassostrea gigas* is the most cultivated shellfish species in the world. Its total standing stock in 2013 was 555,994 t (FAO, 2015). It was introduced in Mexico for commercial purposes in the mid-1970s. The Gulf of California is the most important culture region, having registered a significant increase in the last decade from 407.27 t harvested in 2006, to 3,042 t in 2014 (SAGARPA, 2015). This species is mostly reared in estuarine zones where natural productivity is high, thus ensuring its development. At same time, however, these water bodies are continuously exposed to pollutants from natural and human activities that spill out along rivers leading to the gulf. Some pollutants include heavy metals (Páez-Osuna *et al.,* 1991). Several studies in the Gulf of California indicate that various anthropogenic activities such as mining (Cadena-Cárdenas *et al.,* 2009), agriculture, aquaculture (Páez-Osuna *et al.,* 2003), and urban development (Ruiz-Luna & de la Lanza-Espino, 1999) are sources of diverse heavy metals.

Compared to other coastal areas of the Gulf of California, such as the east coast (Cadena-Cárdenas *et al.,* 2009) and the northern region (García-Rico *et al.,* 2001), the traditional southeastern oyster-culture areas in the state of Sinaloa are relatively more affected by anthropogenic activities, mostly agriculture. Its agriculture production of vegetables and grain crops make it a region of national importance. It is surrounded by some 350,000 ha of agriculture, forest, animal farms, and more than 50 shrimp farms (Honorable Ayuntamiento de Guasave, 2016). Therefore, these areas are under permanent pressure from anthropogenic pollutants from various sources located adjacent to the harvesting areas or at a relatively short distance from where oysters are cultured.

Since oysters are sedentary and filter feeders, they are susceptible to metal accumulation and, therefore, are ideal sentinel organisms for assessing environmental pollution along tropical and subtropical coasts (Páez-Osuna *et al.,* 1995). Further, this bivalve can be a vector of toxic chemicals for humans because is commonly consumed raw. The information on the levels of heavy metals in oyster species from the southeastern coast of the Gulf of California refers to mangrove (*C. corteziensis and C. palmula,* Carpenter, 1857), and rock *C. Iridescens* (Hanley, 1854) wild populations (Páez-Osuna *et al.*, 1991, 1993, 2015). There are few studies on cultured oysters (Osuna-Martínez *et al.*, 2010, 2011; Vázquez-Boucart *et al.*, 2014) from different Sinaloa coastal lagoons have compared metal burdens in both rainy and dry seasons. Nevertheless, no information is available on metal concentrations in *C. gigas* throughout its culture cycle. The presence of high metal levels in cultivated oyster may be an effect of anthropogenic activities, thus indicating a potential risk for human health.

We undertook this investigation to study the status of metal concentrations in cultured Pacific oyster, *C. gigas*, from a farm located along the southeastern Gulf of California during March-December 2011.

MATERIALS AND METHODS

Study area. La Pitahaya Estuary (Figure 1) is located on the southeastern coast of the Gulf of California (25°21'N, 108°38'W), in the state of Sinaloa, Mexico, and is a part of the San-Ignacio-Navachiste-Macapule (SINM) lagoon system. The lagoon system is a marine environment during most of the year due to its two outlets that permanently connect it with the Gulf of California. The lagoon area of SINM has around 22,314 ha, with an estimated population of 91,156 people (Páez-Osuna & Osuna-Martínez, 2015). Mangrove communities surround La Pitahaya Estuary. The climate of the study area is temperate-subhumid with summer rains (INEGI, 2001). The main activity of the study area is intensive agriculture (105,000 ha) characterized by the use of irrigation with the application of high levels of fertilizers and pesticides (Hernández-Cornejo *et al.,* 2005; Páez-Osuna & Osuna-Martínez, 2015). Artisanal fishing and well-developed industrial shrimp fisheries are present (Ruiz-Luna & de la Lanza-Espino, 1999). There are 25 shrimp farms equivalent to 6,621 ha and broiler chickens (77,785 chickens/year, Páez-Osuna & Osuna Martínez, 2015).

Experimental animals. Seven thousand Japanese oyster juveniles (44.78 ± 7.97 mm shell height, SH; 5.56 ± 2.20 g body weight, BW) were cultured in racks suspended in a long-line system (n = 250 oysters/rack). Oysters were acclimated as mentioned by Gallo-García *et al.* (2001) and cultivated according to García-Ulloa *et al.* (2008).

We recorded oyster BW and water quality parameters monthly (March to December 2011). The BW of 50 oysters was measured *in situ* with a portable balance (0.00 g). Water temperature and dissolved oxygen, DO, were determined with an oximeter (YSI 55/12FT, Ohio, USA), salinity with a refractometer (ATAGO, S/Mill), pH by using a pH meter (HANNA, HI 8314, USA), and depth and transparency with a Secchi disk.

Trace metal analysis. Monthly (from March to December 2011), a total of 30 oysters were collected, rinsed with sea water, stored on ice in po-

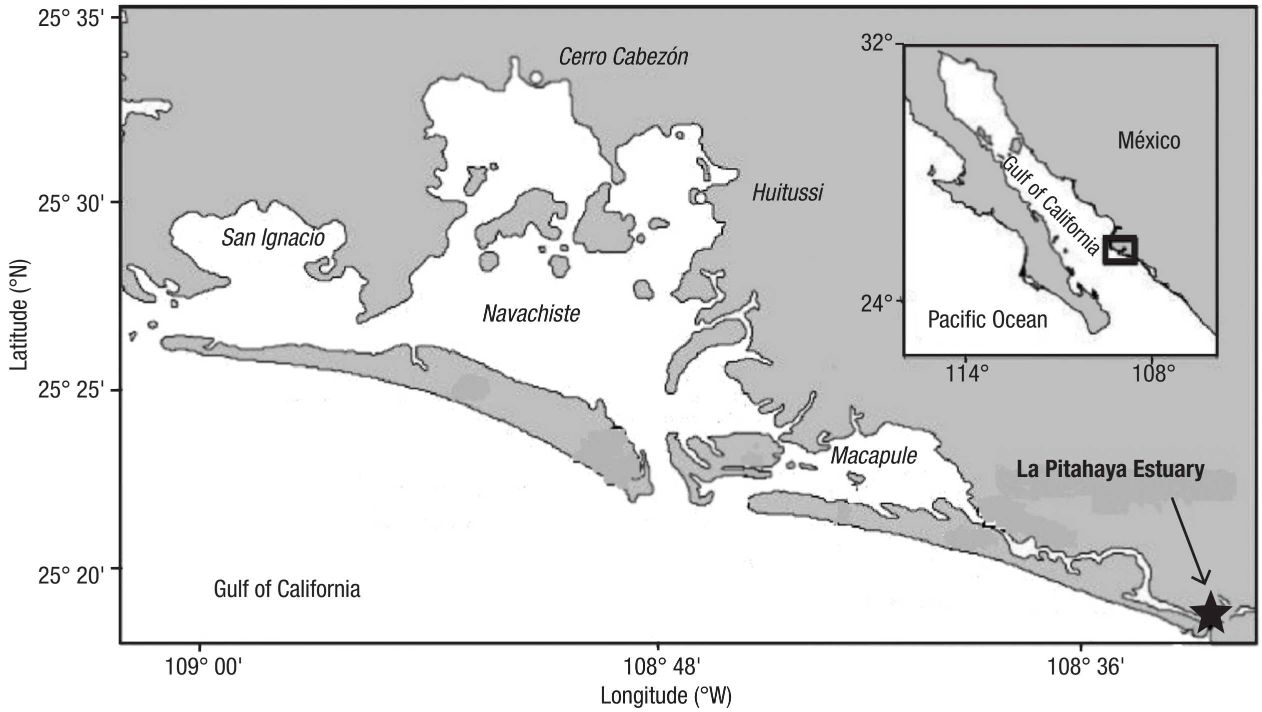

Figure 1. Location of the study area. La Pitahaya estuary (star), part of the San Ignacio-Navachiste-Macapule lagoon system, in Sinaloa, Mexico.

lyethylene plastic bags, and transported to the laboratory for cleaning, sacrificing, and shucking. Thus, a pool of 30 oysters was obtained each month. Oysters were chosen of nearly equal length to limit possible size differences given. Bivalves were opened with a knife to remove soft tissues from the shells and thoroughly washed with double distillated water. Samples were subsequently freeze dried, pulverized in a mortar, and homogenized by quartering, so that all fractions of the sample were equal in composition. The flesh weights and dry weights of the samples were recorded using a digital analytical balance (AE Adam, 0.001 g). To avoid metal residues, we used only high-quality reagents (GR grade, Merck Company). All material used were first cleaned with nitric acid (10%) for a 24 h period and rinsed with double distillated water.

To analyze metal burdens, each month an aliquot (1.27-1.46 g, dry weight) of oyster sample was digested with HNO_3 using a microwave digester (Paar Physica Multiwave Six Place) at 300 W for 5 min, then at 600 W for 10 min. H_2O_2 was then slowly added to the vessels, which were kept in a hood until all bubbling ceased. The digestion procedure proved satisfactory. Digestion of Cu, Cr, Cd, Ni, Pb, Zn, and Hg were performed in 50 mL lined digestion vessels, while As was digested in 100 mL vessels, both equipped with safety relief valves. After digestion, extracts were allowed to cool at room temperature for 25 min and then diluted to 10 ml with de-ionized water. Digests were stored in labelled polyethylene vials at 0-5 °C until we performed the metal analysis.

Cu, Cr, Cd, Ni, Pb, and Zn were analyzed by atomic absorption spectrophotometry (AAS) with flame (Perkin-Elmer, AAnalyst100), As by AAS with hydride generation, and Hg by AAS with cold vapor. For quality assurance, the oyster tissue standard reference material sample (1566b for oysters, National Bureau of Standards, NBS), reagent blanks, and duplicate samples were run with each digestion series. Experimental values for all metals (mean recovery) were in good agreement with the NBS certified values (Table 1). Heavy metal concentrations were calculated based on dry weight (µg g^{-1}). For each element (Cu, Cr, Cd, Ni, Pb, As, Zn, and Hg) the detection limits were 0.032, 0.032, 0.008, 0.46, 0.10, 0.002, 0.039, and 0.0003 µg g^{-1} dry weight, respectively.

Statistical analyses. Descriptive statistics (mean, standard deviation, maximum and minimum limits) were used for metal concentrations each month. The coefficient of variation (CV) of metal burdens was used to test the reliability of data with regard to the effect of parameters and BW. Correlations were made with heavy metal levels, BW, and physic-chemical parameters. Statistical analyses were performed ($p < 0.05$) using the STATISTICA (StatSoft Inc., Tulsa, OK, USA) software package.

Table 1. Analytical results of standard reference oyster material (1566b) in this study (µg g-1, dry wt), developed in La Pitahaya estuary, Sinaloa, Mexico.

Element	Established values	Found values	Mean recovery (%)
Cu	0.723	0.699	96.7
Cr	18.38	20	91.2
Cd	4.78	5	95.6
Ni	19.46	20	97.3
Pb	9.38	10	93.8
As	0.09	0.1	90
Zn	957.5	1000	95.7
Hg	0.025	0.023	93.6

Table 2. Concentration of Cu, Cr, Cd, Ni, Pb, As, Zn, and Hg (dry and fresh weight, μg g^{-1}; mean, standard deviation, maximum and minimum limits) of cultivated oysters from La Pitahaya, Guasave, Sinaloa, Mexico, during 2011.

Month	Cu	Cr	Cd	Ni	Pb	As	Zn	Hg
Mar.	<0.032	5.1	12.5	2	0.8	0.25	138	0.038
	<0.032	**3.52**	**8.73**	**1.39**	**0.55**	**0.17**	**96.36**	**0.026**
Apr.	43.5	0.4	17.5	2.8	3.46	0.48	215	0.003
	29.96	**0.29**	**12.05**	**1.92**	**2.38**	**0.33**	**148.08**	**0.002**
May	26.2	41	11	11	1.25	0.38	170	0.003
	20.58	**32.21**	**8.64**	**8.64**	**0.98**	**0.29**	**133.97**	**0.002**
Jun.	34	2.4	10.5	3.7	2.25	0.4	193	0.008
	23.32	**1.64**	**7.20**	**2.53**	**1.54**	**0.27**	**132.39**	**0.005**
Jul.	70	22.5	21.4	10	2.25	0.44	336	0.0085
	53.15	**17.08**	**16.24**	**7.59**	**1.70**	**0.33**	**255.12**	**0.006**
Aug.	68	106	14	42.6	3.45	0.33	334	0.041
	51.65	**80.52**	**10.63**	**32.36**	**2.62**	**0.25**	**253.74**	**0.031**
Sept.	85	7	19	5	1.8	0.25	418.6	0.02
	59.37	**4.88**	**13.27**	**3.49**	**1.25**	**0.17**	**292.40**	**0.013**
Oct.	62.5	40.5	14	13	4.6	0.3	338	0.0185
	43.04	**27.89**	**9.64**	**8.95**	**3.16**	**0.2**	**232.79**	**0.0127**
Nov.	50.5	23	9	11.5	0.8	0.4	230	0.02
	39.67	**18.07**	**7.07**	**9.03**	**0.62**	**0.31**	**180.71**	**0.015**
Dec.	74.5	1.8	9.5	1	1.2	0.46	242	0.038
	51.10	**1.23**	**6.51**	**0.68**	**0.82**	**0.31**	**166**	**0.026**
Mean	51.42	24.97	13.84	10.26	2.18	0.37	267.42	0.02
	(25.92)†	(32.38)	(4.22)	(12.18)	(1.28)	(0.08)	(92.29)	(0.01)
	37.09	**18.01**	**9.98**	**7.40**	**1.57**	**0.26**	**192.92**	**0.014**
CV %	50.4	129.6	30.4	118.7	58.7	21.6	34.5	50
Limits	85-26.2	106-0.4	21.4-9	42.6-1	4.6-0.8	0.48-0.25	418.6-138	0.04-0.003

*Bold numbers = mean fresh weight basis (μg g^{-1}). †Standard deviation.

RESULTS

The mean water parameters were 26.91 ± 4.43 °C, 6.27 ± 1.27 mg L^{-1}, 33.35 ± 3.98 ups, 7.49 ± 0.48, 1.86 ± 0.29 m, and 0.78 ± 0.26 m for temperature, OD, salinity, pH, depth, and transparency, respectively.

The concentration of each metal studied in *C. gigas* varied with the culture time. The highest values were observed as follows: As (0.48 μg g^{-1}) and Pb (3.46 μg g^{-1}) in April; Cd (21.4 μg g^{-1}) in July; Cr (106 μg g^{-1}), Ni (42.6 μg g^{-1}), and Hg (0.041 μg g^{-1}) in August; Cu (85 μg g^{-1}) and Zn (418 μg g^{-1}) in September. The mean metal concentrations (N = 10) were Cu 51.24 ± 25.29; Cr 24.97 ± 32.38, Cd 13.84 ± 4.22, Ni 10.26 ± 12.18, Pb 2.18 ± 1.28, As 0.37 ± 0.08, Zn 267.42 ± 92.29, and Hg 0.02 ± 0.01 μg g^{-1}, dry weight (Table 2). The metals in the oyster samples showed the following rank order of accumulation: Zn>Cu>Cr>Cd>Ni>Pb>As>Hg.

Correlation analyses for metal levels, BW, and parameters (Table 3) showed a positive correlation of Cd, Ni, Pb, Zn, Cu, and Cr with BW and T °C, but a negative correlation of Cr, Ni, Pb, and Zn with pH and DO. Only the BW/T °C and depth/transparency were positively correlated. Strong correlations were obtained for Cr/Ni, Cu/Zn, and Cd/Zn.

DISCUSSION

The results of metal analysis showed that the concentrations of these eight elements in *C. gigas* reared at the La Pitahaya estuary, Sinaloa, varied monthly. Mean values of Cu and Cd burdens were higher when compared to other studies in Mexico but lower when compared to other countries (Table 4). Some trace metals are essential for normal development of mollusks (Bryan, 1971). Zn, Na, and K are needed for tissue formation and metabolic physiology, while Fe, Cu, and Al are involved in cellular metabolism, protein synthesis, and lipid/carbohydrate metabolism (Barile, 2008). Levels higher than these requirements, however, may cause physiological damage that could threaten growth performance. In this study, oyster BW showed a constant increase until July.

However, with the exception of As and Pb, the remaining metal levels decreased from July to September. Loss of metals in soft tissue could be attributed to a combined effect of water salinity dilution during the rainy season (July to September) and spawning (Robinson *et al.,* 2005; Le *et al.,* 2015), which affect the metal levels by decreasing the concentrations. Jara-Marini *et al.* (2013) mention that Cu levels in adult oysters decrease during the post-spawning event and Lango-Reynoso *et al.* (2010) observed that variations of Cd accumulation in *C. virginica* (Gmelin, 1791) are a result of different stages of reproduction, among other factors. This suggests that oysters accumulated metals as their BW increased until the rainy season, which induced them to spawn and, consequently, caused the BW and metal levels to decrease. In wild populations, fluctuations in the trace metal levels of cultured oysters depend on several factors, such as the season. For instance, Páez-Osuna *et al.* (1995) concluded that levels of some metals vary seasonally with gonad maturation, while Páez-Osuna & Marmolejo-Rivas (1990a, 1990b), and Páez-Osuna *et al.* (1995) found higher levels of Cu and Zn at the end of the reproductive cycle of several oyster species. These previous findings coincide with our results since the higher metal levels were obtained after the oysters reached their highest BW (July-August). As Páez-Osuna *et al.* (1995) determined, some metal concentrations (Cu, Cd, Cr, Ni, Zn, and Hg) during the oyster pre-spawning period (June) were low due to dilution of metals in the soft tissue as the BW increased.

Metal concentrations in *C. gigas* cultivated at La Pitahaya estuary were initially sampled when oysters had a shell height around 35-45 mm (3-4 months after culture started), coinciding with the specimen size collected by Páez-Osuna *et al.* (1991) for analyzing trace metals in different bivalve species. In this study, the ranking of metal concentrations in *C. gigas* reflects the typical metal accumulation of other *Crassostrea* species (Phillips & Muttarasin, 1985; Páez-Osuna & Marmolejo-Rivas, 1990a; Lin & Hsien, 1999), for which Zn posted the highest level and Hg the lowest.

Osuna-Martínez *et al.* (2010) and Ochoa *et al.* (2013) highlighted the importance of oyster depuration prior to being analyzed for heavy metal concentration in order to eliminate digested and undigested food and other particles from gut contents with a potentially high-trace metal burden. This procedure allows us to quantify metal accumulation in body tissue. In our case, *C. gigas* was not depurated for analytical purposes since, first, such a procedure is not a common practice among local farmers and vendors; second, this bivalve is consumed raw.

Different national and international metal regulation for seafood with a fresh weight basis establish the following limits: Cu = 32.5 μg g^{-1} (FAO, 1983), Cr = 13 μg g^{-1} (FDA, 2003), Cd = 0.5 μg g^{-1} (NOM, 1993), Ni = 80 μg g^{-1} (FDA, 2003), Pb = 1 μg g^{-1} (NOM, 1993), As = 80 μg g^{-1} (NOM, 1993), Zn = 718 μg g^{-1} (FDA, 1993), and Hg = 1 μg g^{-1} (NOM, 1993). The values of Cu, Cd, Cr, and Pb surpassed permissible concentrations. The Cd, Cu, Zn, Pb, and As burdens were higher compared to those reported by García-Rico *et al.* (2001) for *C. gigas* cultured on the northwestern Gulf of California coast (0.95, 4.55, 22.75, 0.62, and 0.06 μg g^{-1}, respectively). Also, Cd, Cu, and Pb were higher than the levels obtained by Najiah *et al.* (2008) for *C. iredalei* (Faustino, 1932) cultivated in Malaysia (1.60, 38.9, and 0.17 μg g^{-1}, respectively) and by Ochoa *et al.* (2013) rearing *C. gigas* in Spain (0.5, 38.83, and 0.26 μg g^{-1}, respectively). Yet the levels of Cu, Ni, and Pb were lower compared to those reported by Cadena-Cárdenas *et al.* (2009) (181, 12.2, and 5.8 μg g^{-1}, respectively) for several species of clams and mussels sampled along the Gulf of California. Differences can be attributed to the specific environmental conditions at those latitudes, human activities surrounding the sampled area, and the species studied, among other factors.

García-Rico *et al.* (2001) and Robinson *et al.* (2005) indicate that a consistent association between particular groups of metals may reflect their common metabolic route. Cu, Cd, Ni, and Zn posted the highest correlation values among the metals since they are mostly related with anthropogenic activities (Jara-Marini *et al.,* 2013). Some fertilizers such as Agrinutriente Micromax, Agrinutriente Cu and Agrinutriente Zn, and the fungicides based on zinc ethylene-bis-dithiocarbamate (Zineb) and cooper oxychloride (Cupravit) are commonly used in the region (Páez-Osuna *et al.,* 1993) reflecting their source, since such compounds contain Cu, Ni, and Zn. The SINM lagoon system is connected to the DR 075 and DR 063 Río Sinaloa irrigation districts and receives agrochemicals mainly from agriculture and aquaculture. Escobedo-Urías (2010) estimated that the SINM received 1243.10 t of inorganic nitrogen and 37 t of inorganic phosphorous from anthropogenic activities in 2007. In the same year, Gómez-Arroyo *et al.* (2013) reported that 6500 t of captan (pentachlorophenol-based fungicide) were applied in 5000 ha of cultures, in northern Sinaloa, among other insecticides (chlorpyrifos, Malathion, carbaryl cypermethrin), herbicides (atrazine, paraquat, mancozeb), and fungicides (cupravit, maneb, benomyl). For example, 121737 t of metam-sodium (dithiocarbamate) and cadusafes (organophosphorus) were applied at the tomato plantations in Culiacan Valley, Sinaloa, during the 2011-2012 culture cycle. The agrochemicals are eventually leached from the soil and transported to the coastal zones where oyster farms are located, and therefore oysters could be exposed to these metals. As for *C. corteziensis* in the same lagoon system, Zn

Table 3. Spearman rank order correlations (r) for metal levels (Cu, Cr, Cd, Ni, Pb, As, Zn, and Hg), oyster weight, environmental parameters (T °C, depth, pH, salinity, DO and transparency) and between metals, in cultivated *C. gigas* from La Pitahaya, Sinaloa, Mexico, during March-December 2011.

Metal/BW/Parameters		BW/Parameters		Metal/Metal	
BW *vs.* Cd	0.66	BW *vs.* T °C	0.80	Cu *vs.* Cd	0.64
BW *vs.* Ni	0.57	BW *vs.* DO	-0.85	Cu *vs.* Zn	0.93
BW *vs.* Pb	0.69	T °C *vs.* pH	-0.56	Cu *vs.* Hg	0.64
BW *vs.* Zn	0.58	T °C *vs.* DO	-0.72	Cd *vs.* Pb	0.59
T °C *vs.* Cu	0.63	pH *vs.* Sal.	-0.65	Cd *vs.* Zn	0.75
T °C *vs.* Cd	0.79	DO *vs.* Trans.	-0.67	Cr *vs.* Ni	0.96
T °C *vs.* Cr	0.73	Depth *vs.* Trans	0.62	Cr *vs.* Zn	0.56
T °C *vs.* Ni	0.77			Ni *vs.* Pb	0.55
T °C *vs.* Pb	0.69			Ni *vs.* Zn	0.59
T °C vs. Zn	0.82			Zn *vs.* Hg	0.56
pH *vs.* Cr	-0.59				
pH *vs.* Ni	-0.66				
DO *vs.* Cr	-0.63				
DO *vs.* Ni	-0.61				
DO *vs.* Pb	-0.55				
DO *vs.* Zn	-0.60				

Only significant correlations ($p< 0.05$) are included.

and Cu concentrations in *C. gigas* may be due to the use of Cu- and Zn-based agriculture products (Páez-Osuna & Osuna-Martínez, 2015), as well as the use and application rates of feed additives, liming materials, inorganic fertilizers, and antibiotics applied in the shrimp farms. Lyle-Fritch *et al.* (2006) identified 106 different types of products, and approximately 42 products that are commonly applied at shrimp farms. As, essential element for normal growth and development (Ochoa *et al.,* 2013), Cu is present in aquatic invertebrates and its bioaccumulation can increase with size (Pan & Wang, 2009). The consumption of oysters with high Cu levels can cause irritation, vomiting, and ulcer and kidney damage (ATSDR, 2004). Gorman (1993) indicates that high levels of Cu could even stunt human growth. Cr is an essential micronutrient trace metal that has a similar pattern to Ni and Zn, and, in humans, Cr is an essential part of the glucose tolerance factor (Cheung & Wong, 1992). Its excess, however, may led to diabetes, may lead to diabetes. Cadmium is a nonessential metal for organisms, highly toxic to wildlife, and carci-

Table 4. Metal concentrations ($\mu g\ g^{-1}$d.w.) in wild and cultured oysters from various countries and the Gulf of California, Mexico.

Cu	Cd	Cr	Ni	Pb	Zn	As	Hg	sp., location, year	Reference
17.5-166.3	1.5-7.4	ND	ND	4.1-9.4	245-2304		ND	*Cc*, CLSEGC, 2005	Frías-Espericueta *et al.* (2009)
15.4-216	ND	ND	ND	3.6-7.6	442-1595		ND	*Cc*, CLSEGC, 1997-1998	Páez-Osuna *et al.* (2002)
33.6-44.9	0.2-0.6	ND	ND	0.3-1.9	263-382		0.03-0.08	*Cc*, Estuary Urías Lagoon, 2006	Jara-Marini *et al.* (2008)
1.7-3.5	0.9-4.9	ND	ND	0.4-0.7	ND		0.03-0.04	*Cc*, Bacochibampo Lagoon, Sonora, 2006	García-Rico *et al.* (2010)
23.1-112.6	1.0-9.0	ND	ND	0.3-2.1	226-1745		0.17-0.57	*Cc*, CJSEGC, 2008-2009	Páez-Osuna-Osuna-Martínez (2015)
33.9-46.0	0.27	ND	ND	0.5-1.8	254.3-348		ND	*Cc*, Estuary Urías, 2006	Jara-Marini *et al.* (2009)
		ND	ND		ND	5.2-11.6	ND	*Cc*, CLSEGC, 2008-2009	Bergés-Tiznado *et al.* (2013b)
		ND	ND		36.6-1702		0.2-0.6	*Cc*/*Cg*, Tobari Lagoon, CEGC, NA	Jara-Marini *et al.* (2013)
9.1-58.0	4.9-13.9	ND	ND	0.5-2.1	113-478		ND	*Cg*, CLSEGC, NA	Osuna-Martínez *et al.* (2011)
	4.2-7.3	ND	ND	7.2-9.9	405.5-987.5		ND	*Cg*, ECGC, NA	Vázquez-Boucard *et al.* (2014)
		ND	ND		ND		0.06-0.91	*Cg*, CLSEGC, NA	Osuna-Martínez *et al.* 2010)
		ND	ND		ND		0.23	*Cg*, Guaymas Lagoon, NA	Green-Ruiz *et al.* (2005)
26.2-85.0	9.0-21.4	0.4-06.0	1.0-42.6	0.8-4.6	138.0-418.6	0.25-0.48	0.003-0.04	*Cg*, Estuary La Pitahaya,SEGC, 2011	This study
391.36	2.19	ND	ND	1.14	1972.17	ND	ND	*Cg*, SE England 2007-2008	Bray *et al.* (2015
100	2.0	ND	ND	ND	2237	ND	ND	*Cg*, Arcachon, France, NA	Table 1 of Bragigand *et al.* (2004)
1041	7.5	ND	ND	ND	4964	ND	ND	*Cg*, Gironde Estuary, France, NA	Table 1 of Bragigand *et al.* (2004)
ND	0.01-0.15	ND	ND	0.019	3.7-11.5	ND	ND	*Cg*, Estuary of Lamnyong River, Indonesia, 2013-2014	Sarong *et al.* (2015)

Cc = *Crassostrea corteziensis*; *Cg* = *Crassostrea gigas*; ND = not determined; NA = not available; CLSEGC = coastal lagoons from Southeast Gulf of California; ECGC = East Coast Gulf of California; SEGC = Southeast Gulf of California; CEGC = Central East Gulf of California.

nogenic to humans (Wong *et al.,* 1981) since it tends to accumulate in the liver and kidney (Abbe *et al.,* 2000). Páez-Osuna & Osuna-Martínez (2015) concluded that high Cd levels in the tissue of *C. corteziensis* from SINM could be related to upwelling events, rather than wastes derived from anthropogenic activities, which could partially explain the Cd burdens bioaccumulated by *C. gigas* in this study. In addition, the authors pointed out that closely related oyster species living in the same site can accumulate significantly different levels of metals. Although Pb is known to be a metabolic poison, low concentrations are often observed in shellfish due to the dietary and dissolved lead available for marine invertebrates (Amiard *et al.,* 1986). Higher levels of Pb in oysters can be due to the river runoff and erosion of this metal from the natural bed rock in the region (Soto-Jiménez *et al.,* 2001), as well as human activities such as tourism, fisheries, and untreated urban sewage, i.e., potential pollution sources. In this case, the pollution status of the SINM lagoon system, where La Pitahaya Estuary is located, is well documented, particularly for heavy metals derived from human activities (Hernández-Cornejo *et al.,* 2005; Escobedo-Urías, 2010; Páez-Osuna & Osuna-Martínez, 2015), which helps to explain the results we obtained.

Based on the metal levels obtained, we conclude that cultivated oysters from La Pitahaya Estuary accumulated Cu, Cd, Cr, and Pb above the permissible limits for human consumption during the 2011 production cycle, thus posing a human-health risk. However, the apparent exposure of metals may not involve a consistent intake since individuals consume oysters occasionally. Due to the intense anthropogenic activity and the significant population in the area, the economic importance of the species, and the public-health concerns regarding raw consumption of oysters, we suggest that studies of heavy-metal concentrations in *C. gigas* farmed in this region be carried out periodically. Depuration, moving oyster racks to lower metal levels sites, and use of sterilized water to clean oysters before consumption are also possible strategies in lowering metal contents (Okazaki & Panietz, 1981; Katayon *et al.*, 2007; Wang & Wang, 2014).

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