PROOF

RESEARCH PAPER

Total Mercury in the Mangrove Oyster *Crassostrea corteziensis* of the Subtropical Urías Lagoon (NW Mexico).

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Abstract

Oysters of Urías lagoon (NW Mexico) were collected every second month from November 2012 to September 2013, to determine the variations of their mercury (Hg^{2+}) content and assess their suitability for human consumption. Concentrations were significantly higher in November than in March and May, which indicates that variability does not depend on the alternation of dry and rainy seasons, and is probably related to the reproductive cycle of this species. In view of the low apparent seafood consumption in Mexico and given the low mean Hg^{2+} concentrations (0.07-0.26 $\mu g\ g^{-1}$, dw), consumption of this oyster does not seem to pose risks to the largest part of the population, but the local fishing communities are the ones most likely to be at risk, because of their higher seafood consumption.

Keywords: Mercury, oyster, risk assessment.

Introduction

Mercury (Hg^{2+}) is of particular interest to environmental scientists and toxicologists because it is easily mobilized for natural causes and anthropogenic activities. For this reason it reaches terrestrial and aquatic environments through atmospheric transport and continental runoff. Eventually, it accumulates in coastal waters and sediments (UNEP, 2013) where, due to bacterial activity, inorganic Hg^{2+} may be converted into different organic species, among which methylmercury (MeHg) is the dominant form (Hylander & Goodsite, 2006). This and the available inorganic fraction enter the aquatic food webs, and have the potential to be biomagnified along the food chain (Li *et al*., 2015; Moreno, Fjeld, Deshar, & Lydersen, 2015).

The amount of Hg^{2+} released to the environment in Mexico during 2004 was estimated at 447.8 x10^3 kg. Releases to soils were 185.7 x 10^3 kg and this is largely from gold mine tailings and informal waste deposits. An almost equal amount (185.3 x 10^3 kg) was contained in manufacturing residues such as batteries, sanitary landfills, thermometers and dental works. From this, 50.5 x 10^3 kg was released as atmospheric emissions from Hg-based paints, waste burning and cement plants. The direct input to the aquatic environment was 6.1 x 10^3 kg, mostly from wastewater treatment systems, paints and residues of dental works (Maiz-Larralde, 2008).

The state of Sinaloa releases only close to 2.2% of these discharges (9.8 x 10^3 kg y^{-1}), but its coastal waters are likely to receive Hg^{2+} from other sources, since they are the recipients of several rivers and streams which originate from the metal-rich Sierra Madre Occidental (Frías-Espericueta *et al*., 2014). In particular, the origins of the rivers reaching the central and southern part of the State (San Lorenzo, Elota, Piaxtla, Presidio and Baluarte) lie close or within the gold-producing mining regions 3, 5, 6 and 7 of the neighboring state of Durango (SGM, 2014).

Urías is a subtropical lagoon located on the Mexican mainland, close to the mouth of the Gulf of California. Its inundated area is close to 1800 ha and it is surrounded by 30140 ha of dryland farming. It receives the solid and liquid wastes from five shrimp farms, one thermoelectric power plant, as well as those generated by the commercial and fishing harbor and related services and by industrial activities (mostly fish and seafood processing plants). Additionally, unknown volumes of untreated

urban wastewater of the densely populated surrounding areas are discharged into this lagoon. For this reason, although under continuous heavy anthropogenic impact, it is scarcely affected by agricultural activities (Ochoa-Izaguirre & Soto-Jiménez, 2014), while the remaining lagoons of Sinaloa receive the rainy season runoff from the vast areas dedicated to intensive agriculture of this state (Páez-Osuna & Osuna-Martínez, 2015).

The mangrove oyster *Crassostrea corteziensis* is a filter-feeding bivalve distributed along the Pacific coast from Mexico to Peru; it is common in all NW Mexico coastal lagoons, where this species is harvested year-round. It is also cultured for its good commercial value, and its metal concentration levels have been used for environmental quality assessment of several Mexican coastal lagoons (Frías-Espericueta *et al.*, 2009; García-Rico, Tejeda-Valenzuela, & Burgos-Hernández, 2010).

The aim of this work was to determine the seasonal variations of Hg^{2+} concentrations in the mangrove oyster, *Crassostrea corteziensis* of Urías lagoon, to assess the level of risk generated by human consumption of these oysters, and to compare the state of Hg^{2+} contamination of this lagoon to that of other lagoons of NW Mexico.

Materials and Methods

Sampling was done every two months, from November 2012 to September 2013, and was carried out at three stations located along the lagoon axis (Figure. 1). At each station, 30 oysters of similar size (annual mean length 4.54±0.16 cm) were hand-collected from the mangrove roots of three separate sampling sites located at a distance of approximately 10 m from each other. This gave three separate samples of 30 oysters and a total of 90 oysters station[-1]. Each sample was placed in an individual polyethylene bag and transported to the laboratory, where the 30 oysters of each sampling site were cleaned, measured, shucked, freeze-dried, and ground and homogenized in a teflon mortar. This gave three composite samples for each station. Three 0.5 g aliquots of each sample were pre-digested overnight at room temperature with 5 ml of HNO_3 (trace metal grade), digested in teflon bombs at 120 °C for 4 h and diluted to 25 mL with Milli-Q water (Delgado-Alvarez, Ruelas-Inzunza, Osuna-López, Voltolina, D., & Frías-Espericueta, 2015). Total mercury was determined in a Buck Scientific model 410 mercury analyzer at λ = 253.7 nm, after reduction with $SnCl_2$.

For QA/QC all tissues were analyzed in triplicate, the accuracy of the analytical method was evaluated with the certified reference material DORM-3 with 100.3% recovery. Blanks were used to check contamination and all materials were acid-washed (Moody & Lindstrom, 1977). Detection limit was 0.01 $\mu g\ g^{-1}$.

The hazard quotient (HQ) is used to estimate the potential risk to human health due to the level of exposure to (or of ingestion of) a given contaminant and is calculated with the equation $HQ = E\ R_D^{-1}$ where E and R_D are the level of exposure and the reference (safe) dose, respectively. In the case of total Hg ingestion, R_D for an adult is 0.5 $\mu g\ kg^{-1}\ day^{-1}$ (FDA, 2006) and the level of exposure was calculated as $E = C\ I\ W^{-1}$, where C is the mean total concentration of Hg (in $\mu g\ g^{-1}$, wet weight) of *C. corteziensis* of Urías lagoon, I is the apparent daily consumption and W is the mean weight of target consumers (Newman & Unger, 2002). Mean Mexican oyster consumption is 0.36 kg $person^{-1}\ year^{-1}$ (CONAPESCA, 2013), and W for Mexican adults (18 to 66 years) is 70.7 kg (CANAIVE, 2012).

Data were not normal (Kolmogorov-Smirnov test). Therefore, geographic and time-related differences in Hg content were determined comparing the values obtained on each sampling date at the three sites of each station with a three-way ANOVA test after R1 rank transformation (Conover, 2012). Significantly different means were identified with the Holm-Sidak test.

Metals may be excreted at a slower rate than that of intake. In this case, a direct correlation may be expected between metal contents and age or size (Trudel & Rasmussen, 2006). In this study, the relationship between Hg contents of soft tissues and weight and size of oysters were calculated with Spearman's correlation tests. The level of significance used for all tests was α = 0.05 (Zar, 1999).

Results

Probably because of the low variability of size, no significant relationship was observed between Hg^{2+} concentrations and size or weight (Spearman's ρ=0.02 in both cases, n=45, P>0.5). The mean values determined at the three sampling points of each station were not significantly different, and there were no significant differences between stations (3-ways ANOVAs, P>0.5 in both cases). The only significant differences (P=0.007) were related to the time of sampling, since samples collected in November had higher mean values than those collected in March and May (Table 1).

Assuming an approximate water content of 80%, the mean Hg^{2+} concentration determined in this study (0.148 $\mu g\ g^{-1}$, dry weight= dw) is equivalent to 0.037 $\mu g\ g^{-1}$ wet weight (ww). Using this value and the apparent mean national consumption the calculated HQ value (<0.001) is three orders of magnitude below the level of risk, and remained at this level even using the highest mean value (0.26 $\mu g\ g^{-1}$ dw = 0.065 $\mu g\ g^{-1}$ ww, HQ= 0.0016) detected in September at station 3.

Discussion

Given the lack of difference between sampling sites and sampling stations, the values obtained in this
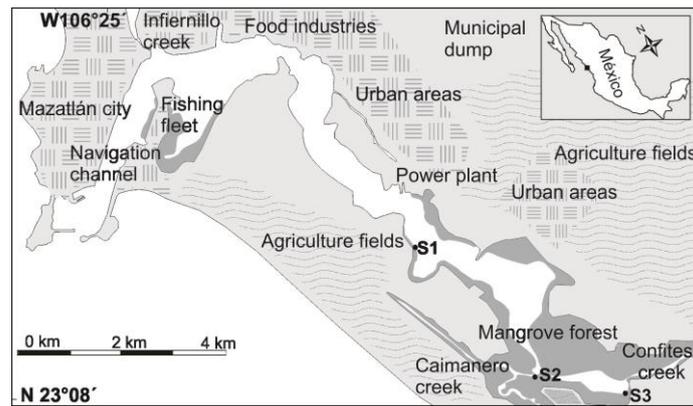
Figure 1. Urías lagoon, location of the sampling stations (S1, S2 and S3), landing site of the traditional fishing fleet and main activities in surrounding areas.

Table 1. Mean (± standard deviation) total mercury content ($\mu g\ g^{-1}$ dw) of *C. corteziensis* collected at three stations of Urías lagoon

Month	Station 1	Station 2	Station 3
Nov	0.23±0.11 [b]	0.20±0.03 [b]	0.16±0.01 [b]
Jan	0.19±0.06 [ab]	0.15±0.02 [ab]	0.14±0.03 [ab]
Mar	0.15±0.09 [a]	0.12±0.01 [a]	0.13±0.03 [a]
May	0.12±0.02 [a]	0.13±0.02 [a]	0.10±0.02 [a]
Jul	0.11±0.01 [ab]	0.19±0.06 [ab]	0.14±0.02 [ab]
Sep	0.07±0.04 [ab]	0.14±0.07 [ab]	0.26±0.02 [ab]

Different letters indicate significant differences (three ways ANOVA after R1 rank transformation, α=0.05). a≤ab≤b and a<b.

work may be considered representative of the Hg^{2+} content of the mangrove oysters of this lagoon, and lie within the range reported in Mexico for this species and for the Japanese oyster *C. giga*s, which is extensively cultured in the Mexican Pacific NW (Table 2). The only exception is the relatively higher concentrations reported for this species in Tobari Lagoon, which receives drainage effluents from the intensive agricultural areas of El Yaqui valley as well as untreated sewage from Ciudad Obregón (300000 inhabitants) (Jara-Marini *et al*., 2013).

Our data are also within the range determined in most other areas of the American tropical and subtropical Atlantic coast, but lower than those determined in the oysters (*C. virginica*) of Términos Lagoon (Aguilar, Montalvo, Rodríguez, Cerón, & Cerón, 2012), which is affected by oil extraction and agriculture-related activities. Higher concentrations were also observed in *C. rhizophorae* collected from a Cuban estuary, and this might probably be due to the emissions of a chlor-alkali plant (Olivares-Rieumont *et al*., 2012) (Table 3).

Most authors who determined the Hg^{2+} content of wild or cultured oysters of Mexican Pacific lagoons found higher contents during the rainy than in the dry season (Osuna-Martínez, Páez-Osuna, & Alonso-Rodríguez, 2010; Aguilar *et al*., 2012; Jara-Marini *et al*., 2013; Páez-Osuna & Osuna-Martínez, 2015) and, although differences were not significant, the same tendency to higher values during the rainy season was

found by Delgado-Alvarez *et al*. (2015) in the oysters collected every three months during an annual cycle at 12 lagoons or coastal sites of the Mexican NW.

This general tendency is probably due to increased wet deposition and continental runoff from the vast agricultural areas surrounding these lagoons during the rainy season (Páez-Osuna & Osuna-Martínez, 2015), while in the case of Urías lagoon contaminant inputs are continuous, and consist of treated and untreated urban wastewater, effluents from shipyards and fish processing plants and fumes and cooling water from an oil-burning power plant (Jara-Marini, Soto-Jiménez, & Páez-Osuna, 2008; Ochoa-Izaguirre & Soto-Jiménez, 2014).

The continuous waste inputs are the most probable explanation of the low variability of our data, which coincides with the narrow range of Hg^{2+} contents determined by Jara-Marini *et al*. (2008) in oysters of Urías lagoon (Table 2). They further explain the lack of significant differences between dry and rainy season, since our data indicate that the only difference was between data obtained in the dry season.

Therefore, rather than showing a difference between the dry and the rainy season, our results indicate a significant difference between the end of the warm season and the early to late spring-early summer season. This seems related to the seasonal variations of the mean gonadic index, which shows a sudden decrease in May, indicating temperature-

Table 2. Mercury contents (µg g^{-1} dw) of *Crassostrea* spp. of NW Mexican coastal waters

Species	Area	Hg^{2+}
C. corteziensis[a]	Coastal lagoons, Sinaloa-Nayarit	0.12-0.19
C. corteziensis[b]	Tobari lagoon, Sonora	0.38-0.53
C. corteziensis[c]	Urías lagoon, Sinaloa	0.03-0.08
C. corteziensis[d]	Bacochibampo Bay, Sonora	0.12-0.16*
C. corteziensis[e]	Coastal lagoons, Sinaloa	0.16-0.58
C. corteziensis[f]	Coastal lagoons, Sinaloa	0.17-0.57
C. gigas[a]	Coastal lagoons, Sonora	0.18-0.25
C. gigas[e]	Coastal lagoons, Sinaloa	0.06-0.91
C. gigas[g]	Coastal waters, Sonora	0.23**
C. corteziensis	Urías lagoon, Sinaloa (this study)	0.07-0.26

[a]Delgado Alvarez *et al.* (2015); [b]Jara-Marini *et al.* (2013); [c]Jara-Marini *et al.* (2008); [d]García-Rico *et al.* (2010); [e]Osuna-Martínez *et al.* (2010); [f]Páez-Osuna and Osuna-Martínez (2015); [g]Green-Ruiz *et al.* (2005). *Calculated from wet weight, assuming 80% moisture; **One sample

Table 3. Mercury contents (µg g^{-1} dw) in oysters of American Atlantic tropical and subtropical areas

Species	Area	Hg^{2+}
Crassostrea virginica[a]	Términos lagoon, SE Mexico	0.20-2.00
Crassostrea virginica[b]	Northern Gulf of Mexico, U.S.A.	0.03-0.5
Crassostrea rhizophorae[c]	Villa Clara, Cuba	0.19-0.69
Crassostrea rhizophorae[d]	Santos and Paranaguá estuaries, Brazil	0.14-0.37
Crassostrea rhizophorae[e]	Gulf of Paria, Venezuela and Trinidad	0.01-0.07
Crassostrea virginica[e]	Gulf of Paria, Venezuela and Trinidad	0.01-0.06

[a]Aguilar *et al.* (2012); [b]Apeti *et al.* (2012); [c]Olivares-Rieumont *et al.* (2012); [d]Torres *et al.* (2012); [e]Rojas de Astudillo *et al.* (2005).

induced mass spawning, followed by a long period of recovery lasting through the winter months (Frías-Espericueta, Páez-Osuna, & Osuna-López, 1997).

Although maternal offloading of contaminants, including Hg^{2+}, has not been described for invertebrates, it is known to occur in several species of teleosts (Sijm, Selnen, & Opperhuizen, 1992; van de Merwe *et al.*, 2011), elasmobranchs (Lyons & Lowe, 2013; Le Bourg, Kiszka, & Bustamante, 2014), dolphins (Yordy *et al.*, 2010) and humans (Ask, Åkesson, Berglund, & Vahter, 2002), and seems a likely explanation for the significantly lower Hg^{2+} content in oysters at the peak of their spawning period.

Present Mexican regulations indicate that the maximum Hg^{2+} content (as MeHg) in marine products other than tuna and related species should not exceed 0.5 µg g^{-1} ww (Secretaría de Salud, 2011), while the maximum limits established by the European Community is 1.0 µg g^{-1} ww for most fish and 0.5 µg g^{-1} for remaining fishery products, including bivalve molluscs (European Union, 2008). These values are higher than the highest concentration detected in this study, which indicates that the consumption of oysters of this lagoon entails only a limited level of risk.

However, the levels of risk assessed using the only available information on fish and seafood consumption (apparent consumption, calculated considering total national landings and the total Mexican population), might give an optimistic estimate of the levels of risk related to heavy metal

ingestion, including Hg^{2+}. For instance, the information of a recent survey in the coastal city of Mazatlán indicated that, although HQ values for Hg^{2+} ingestion remained below the level of risk, they were more than twice higher than those calculated using official data.

This indicates the urgent need of further studies to identify eventual populations at risk, especially in the traditional fishing communities common along the Mexican Pacific coast, where actual yearly consumption of fishery products may be at least one order of magnitude higher than the national apparent consumption (Delgado-Alvarez, 2015).

Acknowledgments

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