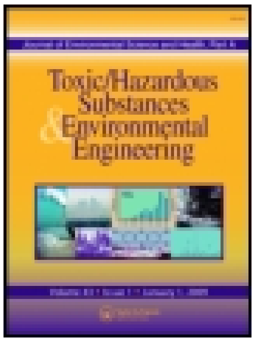

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ABSTRACT

The White and Striped mullets (*Mugil curema* and *M. cephalus)* are highly abundant and commercially important estuarine fish in northwest (NW) Mexico. Because of their feeding habits and habitat, they are likely to accumulate mercury (Hg) in their muscle and liver. The objectives of this study were to determine total Hg distribution in the tissues of interest, to correlate Hg levels between tissues and with length and weight of fish, to estimate the percentage weekly intake (PWI) of total Hg and methyl Hg (MeHg) through consumption of both mullet species, and to compare total Hg levels with mullets from other areas. The highest total Hg concentration (1.031 $\mu g\ g^{-1}$ dry weight) was measured in liver of White mullet. In both species, the order of averaged Hg concentrations (*M. curema* liver 0.272, muscle 0.184; *M. cephalus* liver 0.211, muscle 0.129 $\mu g\ g^{-1}$ dry weight) were liver>muscle. Correlations of Hg in muscle and liver with total weight of *M. curema* were significant. In *M. cephalus*, Hg levels in liver were significantly correlated with total length and weight. The PWI of THg (<4%) and MeHg (<10%) were not elevated for both species. Concentration of Hg in the studied mullets was comparable to levels in similar species from Mexican waters but lower than results in mugilids from other coastal areas.

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Introduction

Consumption of fish provides energy, high quality protein, trace elements, fat-soluble vitamins and essential fatty acids. Fish, as a food item, is part of the cultural traditions of many peoples and, for some populations, fish and fishery products are a primary source of food and essential nutrients, as is the case in estuarine areas of many developing countries, where the fish is obtained from small-scale fisheries. It is known that 90–95% of the small-scale landings are destined for local human consumption.[1] In the southeast (SE) Gulf of California, the mullets are an important component of the landings of small-scale fisheries in the region. In 2013, the mullet fishery was the 8th in importance of landed weight considering all fisheries, with a landed weight of 3,109 metric tons; in the area, tuna, herring and shrimp are the most important fisheries.[2]

Because of their feeding habits in the water-sediment interface they may be suitable for pollution monitoring in coastal waters.[3] In these types of environments, the presence of Hg is known, and one key aspect of Hg cycle is the presence of this element in biota.In aquatic ecosystems, fish are of concern because they tend to accumulate Hg, particularly methylmercury (MeHg), and also because they are often consumed by humans. Within the frame of the Minamata Convention, Article 19 states that research and monitoring of Hg in fish are relevant in view of ecotoxicology and human health issues.[4]

Though mercury (Hg) occurs naturally in the environment, certain areas of the globe are enriched in comparison to average values of the earth crust. In the United States of America, an Hg mineral belt is located in the California Coastal Range where Hg is produced as a by-product from deposits of gold-silver;[5] this area is relatively close to the Mexican boundary. With respect to anthropogenic emissions of Hg, since colonial times more than 145,000 tons of Hg were carried from Spain to Mexico for gold mining, this long-term activity (over 300 years) resulted in soils that are currently enriched, such areas are known as "hot spots," and they contain above 10 ppb of Hg in the top layers.[6] According to CENICA,[7] overall releases of Hg in Mexican states place Sinaloa (SE Gulf of California) in the 8th place from a total of 32. The above information serves to set in context the complexity of Hg biogeochemical cycle and accordingly, the approaches to monitor the occurrence of this element in the diverse environmental compartments. The use of fish to monitor Hg has several advantages over water and sediments, bioavailability being one of the most relevant issues.

Diet is the main route of Hg uptake in fish, if striped mullet has a trophic level of 2.5 whilst the white mullet has a trophic level of 2.0,[8] we hypothesized that both species have increased concentrations of Hg because of their feeding habits and habitat use, and also that Hg levels are more elevated in the striped

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mullet. Considering the above hypotheses, total Hg was measured in the striped and the white mullets with the aim of knowing: a) the correlation of total Hg in muscle and liver in both species; b) the variation of Hg concentrations in the analyzed tissues with fish dimensions; c) the percentage weekly intake (PWI) of total Hg and MeHg, considering the average fish consumption in the Mexican population, the provisional tolerable weekly intake of total Hg and MeHg and the total Hg concentration in the edible portion of analyzed fish; and d) the comparison of total Hg levels with mugilids from other areas.

Materials and methods

Adult individuals of the white mullet and the striped mullet were collected by local fishermen between December 2010 and June 2011 at the estuarine system of Huizache-Caimanero (Fig. A1). Huizache-Caimanero is located in the southern part of the coastal plateau of Sinaloa, between the rivers Presidio and Baluarte. Ecologically, this system is a marine seasonal floodplain with two basins, Huizache located in the north and Caimanero in the south. The basins are separated by a narrow channel of about 250 m. According to Lankford [9] this is a III-A coastal lagoon with a total area of approximately 260 km^2, but during the dry season the area can be reduced due to elevated temperatures that favor evaporation. The climate is warm, with temperatures ranging from 10.5°C to 36°C.[10] The total area of crops accounts for 37,667 ha.[11] According to the Convention on Wetlands (RAMSAR), around 20,000 birds of 23 species inhabit in this area.[12] Mangrove forests are only located along channels in the south and north parts that connect with the Presidio and Baluarte rivers. These channels are inhabited by red mangrove, white mangrove and black mangrove (*Avicennia nitida*). In both lagoons the mangrove forests are absent.[13,14] The area surrounding this water system also has aquaculture ponds and small towns.[15] The fish were collected during morning hours and high tide with gillnets of 3.5 inches of mesh size, and 100 m long. The catches were placed in separate labeled plastic bags in an icebox. After fieldwork, specimens were transported to the laboratory for taxonomic identification, total length measurement and fresh weight determination (Table 1).

Glassware and other utensils were acid washed using the procedure of Moody and Lindstrom.[16] A portion of muscle tissue from the median dorsal area and the liver were extracted from every individual. Muscle and hepatic samples were kept at low temperatures (around −19°C) until laboratory processing and metal analysis. Processing of samples included freeze-drying (−50°C; 54 × 10^{-3} mbar; 72 h) in a Labconco equipment; grinding and homogenization in agate mortars (Fisher-Scientific, Waltham, MA, USA); and digestion of powdered samples with concentrated nitric acid (JT Baker, Center Valley, PA, USA; trace metal grade) in capped vials (Savillex, Eden Prairie, MN, USA) on a hot plate (Barnstead Thermolyne, Ramsey, MN, USA) at 120°C during 3 h. The acid digestion decomposes the tissue samples and converts organic Hg species into the inorganic form; the inorganic Hg is reduced to the elemental form with tin chloride, and cold vapor is measured. Analyses of total Hg were made by cold vapor atomic absorption spectrophotometry [17] in a Buck Scientific equipment. Conversion of Hg concentration from dry weight (Hg_{dw}) to fresh weight (Hg_{fw}) was made according to Eq. (1):[18]

$$Hg_{fw} = Hg_{dw} \times (100 - \%\ humidity)/100 \qquad (1)$$

The mean percentage of humidity in the edible portion (muscle) was 70%.

The PWI of Hg was estimated according to Eq. (2):

$$PWI = 167 \times Cmc/PTWI \qquad (2)$$

where 167 (g/week) is the individual weekly intake of fish in Mexico (considering an average fish consumption in Mexico of 9.01 kg per capita per year), Cmc is the total Hg or MeHg concentration in fish muscle (mg kg^{-1}), PTWI [19] is the provisional tolerable weekly intake of total Hg (5 μg kg^{-1} body weight) and MeHg (1.6 μg kg^{-1} body weight). In the case of MeHg, estimation was made considering 95% as the amount of MeHg with respect to total Hg in muscle tissue of the mugilid *Liza aurata*.[20] The accuracy and precision of the method was assessed by analyzing reference materials (Table 2). The limit of detection was 0.013 μg g^{-1} dry weight. Blanks and reference materials were run with every batch of 16–20 samples. Results are expressed as μg g^{-1} on a dry weight (dw) basis. Results of total Hg were tested for normality and homoscedasticity; comparisons of average Hg levels between fish species and between tissues of every species were made with a Student's t-test. For both mullet species, levels of Hg in muscle were correlated with corresponding concentrations in liver. Similarly, Hg levels in muscle and liver were correlated with length

Table 1. Average total length and total fresh weight (minimum and maximum values in parenthesis) of collected fish from Huizache-Caimanero lagoon.

Species	Common name	N	Total length (cm)	Total weight (g)
Mugil curema	White mullet	58	24.8 ± 1.7 (21.0–29.0)	143.5 ± 31.6 (95.0–260.0)
Mugil cephalus	Striped mullet	7	25.5 ± 1.2 (24.0–27.0)	148.7 ± 21.3 (121.0–176.0)

Table 2. Results of analytical quality control through Hg determinations in certified reference materials.

Matrix	Code	n	Certified concentration (μg g^{-1})	Measured concentration (μg g^{-1})	Percentage recovery
Fish muscle	DORM-3	8	0.382 ± 0.060	0.351 ± 0.049	92
Fish liver	DOLT-4	7	2.58 ± 0.22	2.60 ± 0.23	101

Table 3. Total mercury (THg) and methylmercury (MeHg) concentrations (μg g^{-1} dry weight) in muscle and liver of mullets *M. curema* and *M. cephalus* from Huizache-Caimanero lagoon and percentage weekly intake (PWI) of THg and MeHg in the edible portion.

Species	Common name	Tissue	N	THg	PWI (THg)	MeHg[a]	PWI (MeHg)
Mugil curema	White mullet	Muscle	58	*0.184 ± 0.140 (0.024–0.773)	3.1	0.175	9.14
		Liver	58	*0.272 ± 0.214 (0.065–1.031)			
Mugil cephalus	Striped mullet	Muscle	7	0.129 ± 0.051 (0.053–0.213)	2.2	0.123	6.42
		Liver	7	0.211 ± 0.137 (0.085–0.509)			

*For a single species, it indicates that tissue concentrations were significantly different ($P < 0.05$).
[a]Estimated as 95% of THg concentration.

and weight of fish. Statistical analyses were made with a specialized software (GraphPad Prism 4.0; GraphPad Software, San Diego, CA, USA) at a significance level $P < 0.05$.

Results and discussion

The highest Hg concentration (1.031 μg g^{-1} dw) was measured in liver of *White mullet*, and the lowest (0.024 μg g^{-1} dw) corresponded to muscle of the same species. Average Hg concentrations in liver of *White mullet* (Table 3) were significantly ($P < 0.05$) higher than in muscle. For both species, the sequence of Hg concentrations was liver>muscle. After Hg uptake by fishes, it is transported all over their body. In fish liver, proteins of low molecular weight (metallothionein-like) are abundant; this may be related to the higher Hg levels in liver than in muscle.[21,22] In this sense, liver is considered as a target organ for Hg because of its direct participation in the element storage and detoxification.[23] Although fish liver has the capacity to control Hg toxicity, this mechanism has a limited effectiveness; i.e. beyond certain amounts of Hg there may be deleterious effects at cellular level.[24]

The correlation of Hg levels in liver with the corresponding values in muscle was significant ($P < 0.05$) in both species (Fig. 1); i.e. there was a trend to increase Hg concentrations in muscle as levels in liver are becoming higher. The differential elemental levels in fish tissue have received considerable attention. Increased Hg concentrations in liver have been attributed to the role of this organ in fish; among the most important functions are detoxification, storage, and transformation of pollutants.[25] Another reason that may define a different degree of Hg accumulation in muscle and liver is the quality of the fish environment. Several authors [26,27] have found that fish from polluted sites tend to accumulate more Hg in the liver; on the contrary, in fish from lightly polluted areas the muscle is the target tissue. The issue of increased concentrations of metals with age has been a matter of debate; though age was not determined, in *M. curema* the levels of Hg in liver and muscle increased significantly with total weight (Fig. 2c and d) but not with total length. This is consistent with the findings of Cresson et al.[28] in the red mullets *Mullus barbatus* and *Mullus surmuletus* from several places in the French Mediterranean; the authors considered that length of fish was not a good indicator of Hg pollution. In the case of *M. cephalus* there was a different behavior of Hg concentrations; in this species elemental levels decreased with fish dimensions (Fig. 3b and c). Even if mullets studied here belong to similar species and were collected in the same area, diverse metabolic and ecological factors might account for the contrasting patterns of Hg accumulation. It has been widely recognized that fish diet constitutes the main source of Hg; perhaps the different food items preferred by these two species turn into a differential Hg accumulation. Another reason might be growth dilution or habitat preference during the life cycle of *M. cephalus*.

With respect to the PWI, values for both species (Table 3) were low (<4%); this percentage is comparable to a study with the blue tilapia *Oreochromis aureus* [29] from a dam in NW Mexico (average PWI = 3.6), to a study with tunas *Thunnus albacares* (PWI = 0.86) and *Katsuwonus pelamis* (PWI = 1.14) from the Eastern Pacific Ocean [30] and to a study [31] in Jamaica with the lionfish *Pterois volitans* (average PWI = 4.0). It is known that MeHg is the most toxic form of Hg; in the marine environment, MeHg is mainly found in the muscle tissue of fish, which is the portion that is usually consumed by humans. In this context, it is relevant

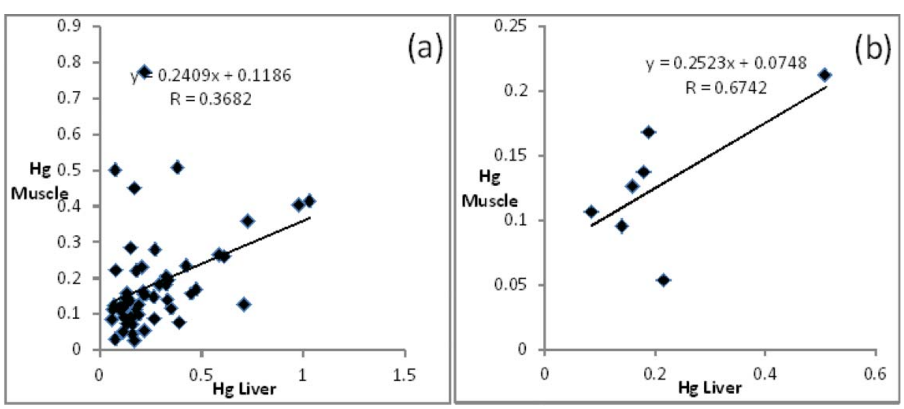

Figure 1. Correlation of Hg concentrations (μg g^{-1} dry weight) in liver and muscle of (a) *Mugil curema* and (b) *M. cephalus* from Huizache-Caimanero lagoon.

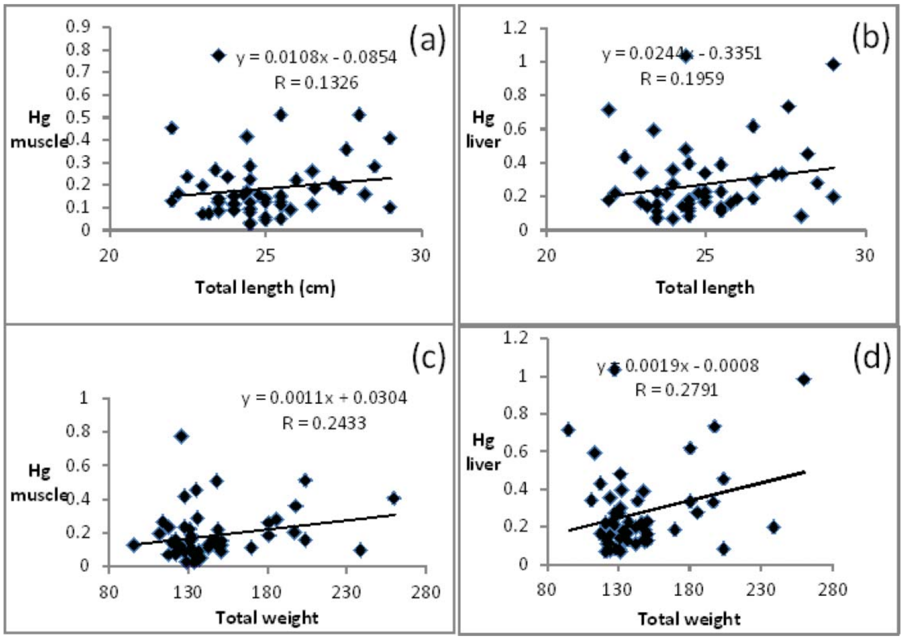

Figure 2. Variation of Hg concentrations (μg g^{-1} dry weight) in (a) muscle (b) and liver with total length and variation of Hg concentrations in (c) muscle and (d) liver with total weight of *Mugil curema* from Huizache-Caimanero lagoon.

to monitor levels of MeHg and their respective PWI. It can be seen that the PWI of MeHg in both mullets were almost three times higher in comparison to the corresponding values of THg. Though values of PWI were low (THg 2.2–3.1%; MeHg 6.42–9.14%), more research is necessary in relation to fish consumption rates because certain sectors of the Mexican population consume much more fish than the average citizen; another issue of interest in terms of the toxicology of Hg relates to the antagonistic role of Se.

A comparison of Hg levels in muscle tissue of several mugilids is presented in Table 4. Information corresponds to ichthyofauna from Mexico and overseas, a total of nine species were considered.[32-48] It was decided to compare only taxonomically related species because similar species show similar physiology and this turns into similar responses to trace metal loads. The lowest Hg value (0.03 μg g^{-1} dw) was recorded in *M. cephalus* from Dakar, Senegal. With respect to the highest concentrations (3.33 μg g^{-1} dw), they were reported in striped mullet from the Chennai estuary (India). As can be seen in Table 4, a high variability (above a hundred times) of Hg concentrations is reported in Table 4. The issue of monitoring metal occurrence in aquatic ecosystems through biota has the advantage (in comparison to surficial sediments and water) of showing the bio-available fraction of the element of interest

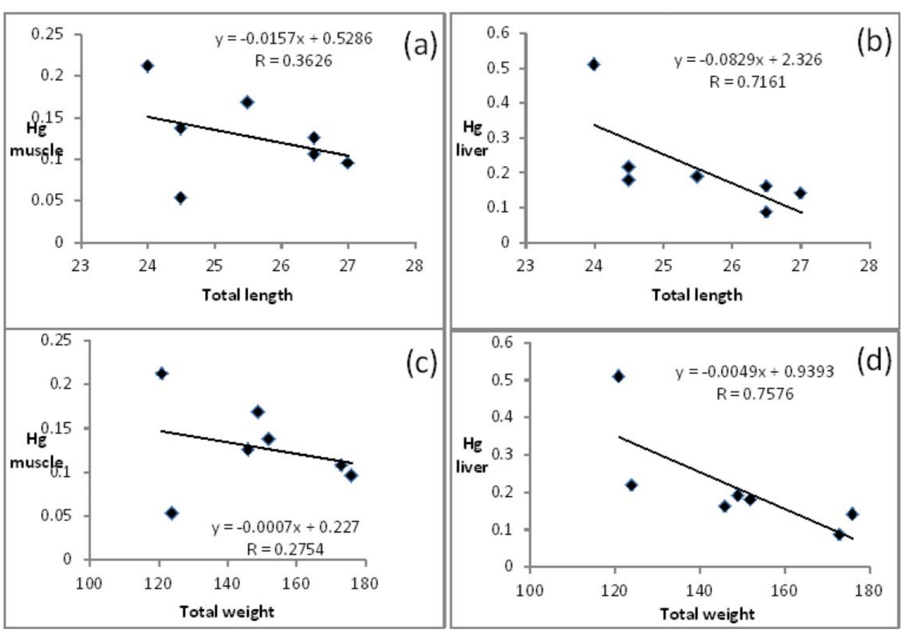

Figure 3. Variation of Hg concentrations (μg g^{-1} dry weight) in (a) muscle and (b) liver with total length and variation of Hg concentrations in (c) muscle and (d) liver with total weight of *Mugil cephalus* from Huizache-Caimanero lagoon.

Table 4. Total mercury levels (μg g^{-1} dry weight) in muscle tissue of mullets (Mugilidae) from diverse sites.

Species	Common name	Hg	Site	Reference
Mexico				
Mugil spp.	Mullet	0.16	Urías lagoon, Sinaloa	[32]
Mugil curema	White mullet	0.47	Coasts of Sinaloa	[33]
Mugil cephalus	Striped mullet	0.07	Coasts of Sinaloa	[33]
Mugil curema	White mullet	0.18	Huizache-Caimanero	This study
Mugil cephalus	Striped mullet	0.13	Huizache-Caimanero	This study
International				
Mugil brasiliensis	Lisa	1.33	La Plata river estuary	[34]
Mugil liza	Mullet	0.04	Guanabara bay, Brazil	[35]
Mugil liza	Mullet	1.33	Argentina	[36]
Mugil auratus	Golden grey mullet	0.13	Mediterranean Sea	[3]
Mugil labrosus	Lesser grey mullet	0.17	Mediterranean Sea	[3]
Mugil capito	Grey mullet	0.17	Mediterranean Sea	[3]
Mugil cephalus	Grey mullet	0.034	Pearl river estuary, China	[37]
Mugil cephalus	Grey mullet	0.34	Ghar El Melh lagoon, Tunisia	[38]
Mugil cephalus	Grey mullet	0.13	Kerala, India	[39]
Mugil cephalus	Grey mullet	0.10	Mediterranean Sea	[40]
Mugil cephalus	Grey mullet	1.20	Mediterranean Sea	[41]
Mugil cephalus	Grey mullet	0.40	Turkey	[42]
Mugil cephalus	Grey mullet	0.70	India	[43]
Mugil cephalus	Grey mullet	0.66	Northeast Mediterranean Sea	[44]
Mugil cephalus	Grey mullet	3.33	Chennai estuary, India	[45]
Mugil cephalus	Grey mullet	<0.083	Chi-ku lagoon, Taiwan	[46]
Mugil platanus	Tainha	0.04	Southern Brazil	[47]
Mugil cephalus	Flathead mullet	0.03	Dakar, Senegal	[48]

and also the integrated values of contamination considering time and space. In the specific case of mullets, it has been documented that they are efficient indicators of Hg contamination.[28]

Conclusion

As concluding remarks, we may say that distribution of Hg in tissues of studied mullets showed that liver accumulated more Hg than muscle; nevertheless, there was a high variability of elemental concentrations in White mullet and Striped mullet. For both mullet species, levels of Hg in muscle were significantly correlated with the corresponding values in liver. In *M. curema*, Hg concentrations in muscle and liver were significantly correlated with total weight. In *M. cephalus*, Hg concentrations in liver were significantly correlated with length and weight of specimens. In relation to the PWI of total Hg and MeHg through consumption of the edible portion of analyzed fishes, estimated values in the scenario with MeHg were three times higher than with THg. PWI values were below 10% and they are not of concern for human health risk. With respect to the comparison of Hg levels in mugilids from diverse coastal areas, our results were comparable to Hg concentrations in Mexican waters but lower than levels in fish from Argentina, India and the Mediterranean sea.

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Appendix

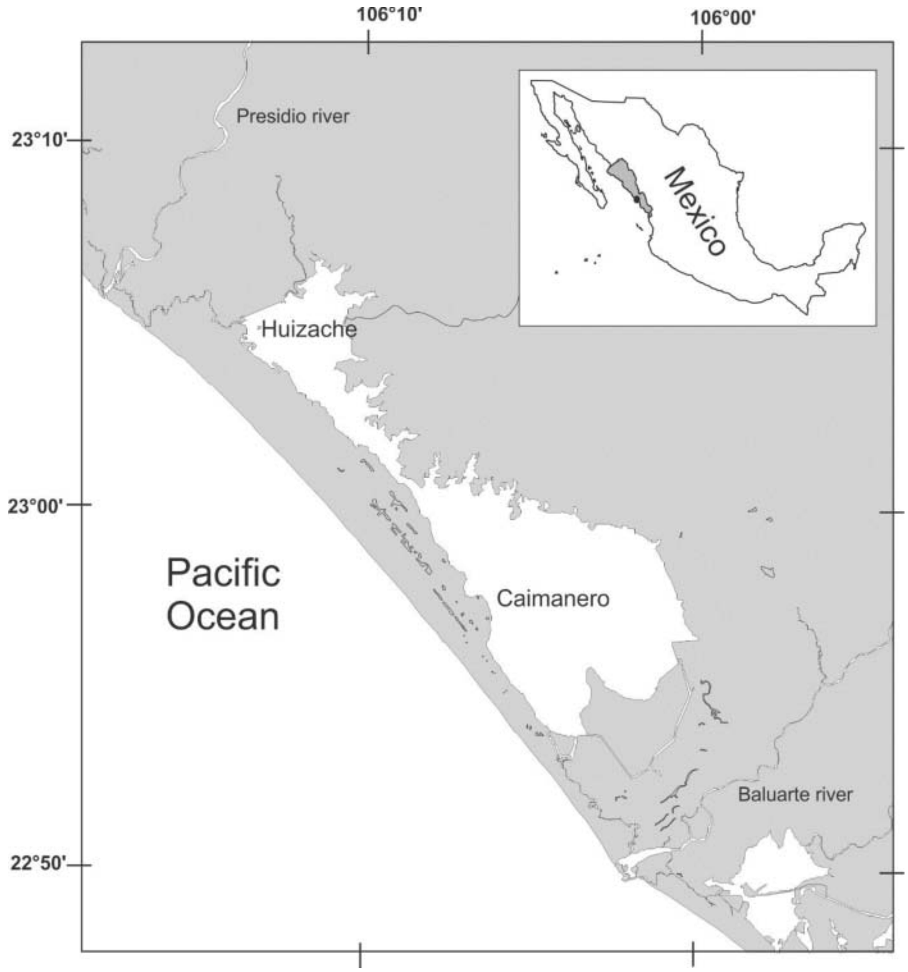

Figure A1. Location of Huizache-Caimanero lagoon in NW Mexico.