

Cd, Cu, Hg and Pb, and organochlorines pesticides in commercially important benthic organisms coastal lagoons SW Gulf of Mexico

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Abstract: The objective of this study was to determine the concentrations of insecticides (DDT and their metabolites) and heavy metals (Cd, Pb, Cu and Hg) in benthic organisms such as oysters, crab and shrimp. The studies were carried out in six lagoon systems along the Gulf of Mexico: Madre, Tamiahua, Grande, Mandinga, Alvarado and Mecoaacán. The sampling sites in each lagoon system were selected in the areas of commercial fishing. The levels of pesticides in oysters and crabs indicated they were in constant contact with these compounds, which are used in livestock, agricultural and aquacultural activities, and in the efforts carried out in adjoining zones to control the vectors of dengue and malaria. The concentration ranking of heavy metals in *F. aztecus* in the Tamiahua system was Cu > Cd > Pb, while for *L. setiferus* in the Alvarado system it was Pb > Cd > Hg.

Keywords: metals; insecticides; shrimp; crab; oyster; Gulf of Mexico

1. Introduction

According to the FAO (2010), global fishing production is 90 million tons and is composed mostly of benthic organisms such as crustaceans, molluscs and fish. In Mexico, the primary fishery resources, by capture volume, are crustaceans and bivalve molluscs, harvested from coastal lagoon systems along the Gulf of Mexico. These, have been negatively impacted by anthropogenic activities such as agriculture, mining, fossil fuel extraction and refinement, and agrochemical industries (Botello & Villanueva, 2010). Such activities have and continue to alter the balance in these aquatic ecosystems. Additional causes of altered equilibria are the introduction of exotic aquatic species, rapid population growth, changes in soil use, and climatic variability (Villanueva et al., 2010). According to the UN (1992), approximately half of the world population lives less than 60 kilometers from the coast and it is predicted that this level will reach 70% by the year 2020. Such coastal aquatic systems integrate wetlands, lagoons, marshes, mangrove swamps, estuaries and river and riparian areas, and systems that receive freshwater, dissolved solids, and particulates and organic and inorganic compounds coming from the continent (Escobar, 2002). As well, they are subjected to the influence of contaminants such as hydrocarbons, heavy metals, organochlorine pesticides and microorganisms (Botello et al., 2002). Such compounds, especially those of synthetic

origin that are resistant to natural processes of degradation in the environment, are very stable and have a long half-life, cause damage to the health and quality of the bioaccumulating organisms that inhabit these systems and, consequently, negatively affect human health (Allsopp & Erry, 2000; Luna et al., 2002; Martínez & Yarto, 2004). Another group of chemical elements that affect aquatic organisms and the environment are the heavy metals. The primary sources of origin along the coastal region of the Gulf of Mexico are fossil fuel extraction and refining, fertilizer production, mining activities and the discharge of domestic sewage effluents (Páez, 2005). The heavy metals turn toxic when they reach levels capable of damaging the vital functions of aquatic organisms, and their toxicity in the ecosystems depends on the degree of oxidation and the chemical form in which they are found in the environment (Priju & Narayana, 2007).

The Gulf of Mexico is one of the most important and productive estuarine zones in the world due to its high biodiversity (Tunnell et al., 2004; Toledo, 2005). Therefore, the concentration of organochlorine pesticides and heavy metals in aquatic organisms of commercial importance from the lagoon systems along the coast of the Gulf of Mexico were evaluated.

2. Materials and Methods

2.1 Study Area

The study was performed in the lagoon systems of Madre, Tamiahua, Grande, Mandinga, Alvarado and Mecocacán, located along the coast of the Gulf of Mexico (Fig 1).

2.1.1 *Laguna Madre*

The lagoon is located in the state of Tamaulipas, Mexico, between the coordinates 24° 01' and 25° 58' N and 97° 23' and 97° 54' W. It is bounded to the north with the Bravo River delta and to the south with the Soto La Marina River estuary. The lagoon covers approximately 200,000 ha and its depth varies between 1.5 and 4.5 m (Carta Nacional Pesquera, 2004; Estavillo & Aguayo, 2012).

2.1.2 *Laguna Tamiahua*

This lagoon system is located in the state of Veracruz, Mexico, between the coordinates 21° 06' N and 97° 23' and 97° 46' W. It is bounded to the north with the Pánuco River and to the south with the Tuxpan River (Castañeda & Contreras, 1994). It covers approximately 88,000 ha (Contreras, 2001; Carta Nacional Pesquera, 2004).

2.1.3 *Laguna Grande*

This lagoon is located in the municipality of Vega de Alatorre in the state of Veracruz between the coordinates 20° 02' and 20° 06' N and 96° 38' and 96° 41' W. It covers a total of 2,250 ha (Carta Nacional Pesquera, 2004).

2.1.4 *Laguna de Mandinga*

This lagoon complex is located in the state of Veracruz between 19° 00' and 19° 06' N and 96° 02' and 96° 06' W; 18 km from the port city of Veracruz (Contreras, 1985). It covers an area of 3,250 ha (Carta Nacional Pesquera, 2004).

2.1.5 *Laguna de Alvarado*

This lagoon system is located between the coordinates 18° 46' and 18° 42' N and 95° 34' and 95° 58' W at 10 m above sea level and 70 km southeast of the port city of Veracruz. It covers an area of

6,200 ha and has a maximum depth of 4.5 m (Reguero & García-Cubas, 1989; Carta Nacional Pesquera, 2004).

2.1.6 Laguna de Mecoacán

This lagoon is located along the coast of the municipality of Paraíso, in the state of Tabasco (Carta Nacional Pesquera, 2004), between the coordinates 18° 16' and 18° 26' N, and 93° 04' and 93° 14' W (Galaviz et al., 1986), and covers an area of 5,168 ha.

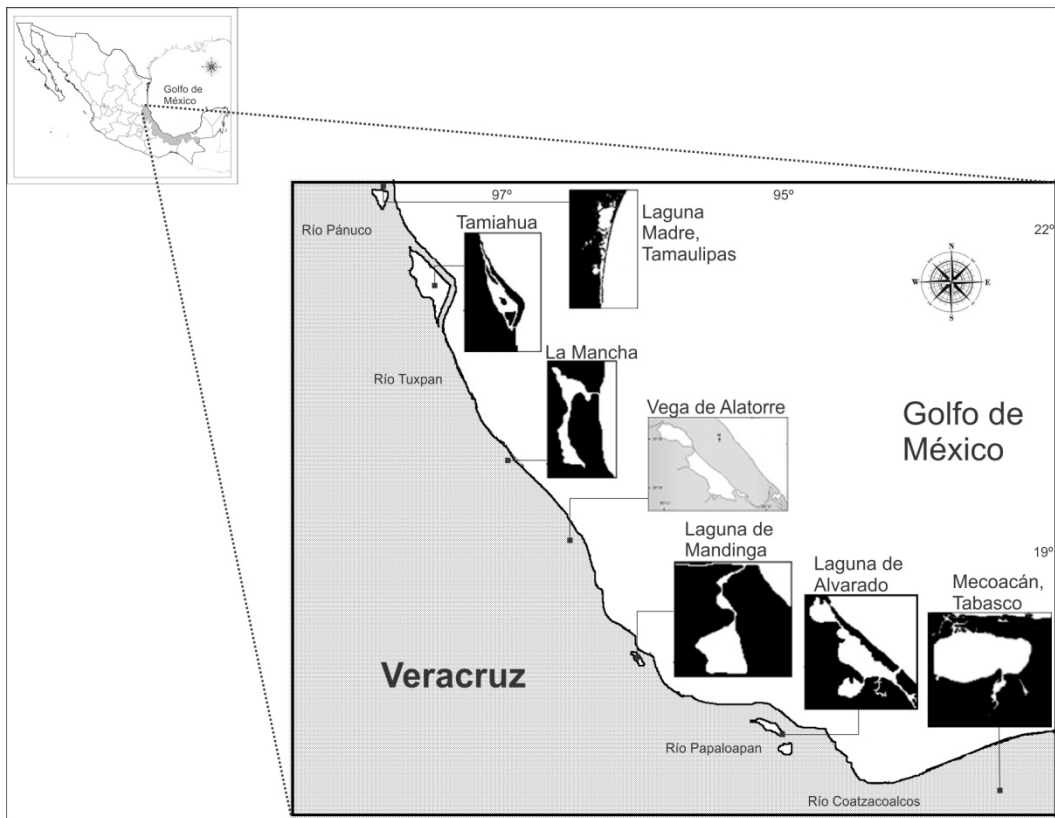


Figure 1 Study area and coastal lagoon systems along the Gulf of Mexico

2.2 Sample Collection and Processing

Oyster, shrimp and crab samples were collected during winter (January and February, a portion of the windy season), during the dry season when water levels are low (March to June), and during the rainy season (July to October). Oyster (*Crassostrea virginica*) samples were collected in 2009 from the lagoon systems of Madre, Mandinga and Mecoacán. Samples of the sharptooth swimming crab, *Callinectes rathbunae*, were collected in 2008 from Laguna Grande. Samples of the shrimp *Farfantepenaeus aztecus* and *L. setiferus* were collected in 2004 and 2010 from the Tamiahua and Alvarado lagoons, respectively. Each oyster sample was composed of 100 oysters, collected by free-diving, at sampling stations in each lagoon system, from which 30 oysters of commercial size were selected (7.0 ± 3.0 cm).

For crabs, the sample was composed of 30 individuals of commercial size (± 110 mm). The crabs were selectively collected using the "nasa" method, which consists of a 0.75 m diameter ring trap

outfitted with 25 mm plastic netting set one day before collection; a method assuring the capture of commercial sized crabs. For shrimp, 1 kg of commercial sized specimens (10 – 15 g per specimen) was collected in each sample.

The oyster, shrimp and crab samples were cleaned to remove any adhering materials or particles. In accordance with NOM-109-SSA1-1994 (Diario Oficial de la Federación, 1994), the samples were then placed in labeled polyethylene Ziplock® bags and stored in refrigerators at 5 °C prior to transportation to the laboratory for analysis.

2.3 Laboratory Analysis

2.3.1 Sample Preparation for Oysters, Crabs and Shrimp

2.3.1.1 Oysters

The oysters were shelled and the soft tissues were separated by dissection and placed in labeled Ziplock® bags, in triplicate, for sample management. The samples were stored at -40 °C in a Thermo Model 726 ultrafreezer (Thermo Fisher Scientific Inc., San Fernando, CA, USA). The frozen samples were lyophilized in a Thermo Savant Moduly OD-114 for 72 hours at -49 °C and at a vacuum pressure of 36×10^{-3} mbar. The samples were then stored in hermetically sealed bags inside a dessicator with silica gel to control humidity. The samples were then ground in an Osterizer blender until obtaining a fine particle size and then they were homogenized with a N° 30 sieve with a mesh size of 595 µm. To avoid contamination from humidity, the samples were stored in a dessicator for approximately 3 days. For heavy metal determination, the samples were digested, and extraction was used for insecticides. In both analyses a microwave was utilized (CEM model MARS 5, CEM Corporation, Matthews, North Carolina, USA), following the Oyster Pure method (EPA, 2000).

2.3.1.2 Crab

The crab were dissected to separate the muscle from the viscera and to obtain two combined samples of approximately 500 g wet weight each, which were then stored in Ziploc® bags. The storage techniques, dehydration and grinding of the samples, as well as the analyses for the determination of organochlorine pesticides were the same as those used for oysters.

2.3.1.3 Shrimp

The specimens in each sample were dissected to obtain one composite sample of the tail muscle. These samples were obtained in triplicate. Sample storage, dehydration and grinding, as well as the determination of heavy metals were performed using the same techniques and methods as with oysters.

2.3.2 Processing the Oyster and Crab Samples for Pesticide Determination

The materials used in the study were prepared according to the analytical protocol for pesticide residues (Waliszewki et al., 1985). The glassware was washed with potassium dichromate and rinsed with tap water, followed by a rinse with distilled water (Milli-Q), petroleum ether and acetone. All solvents and reagents used were analytical grade. To avoid any cross-contamination of the samples, the purity of the petroleum ether used to wash the glassware was evaluated periodically using gas chromatography. The samples were analyzed using hexane (Baker) with a boiling temperature range of 40-50 °C, sodium sulphate powder (Baker) previously activated and purified in a forced air oven (Riossa CF-102) at a temperature of 650 °C for 16 h, and with sulfuric acid (Merck) of 95 to 97% purity. For quality control, the chromatograph readings for each pesticide

were adjusted to follow the calibration of a 5-point curve. Reference samples were used to construct the calibration curve using a ChemStation HP 3398A (ChemService, Inc., West Chester, Pennsylvania, USA). To guarantee a recovery of 93%, a calibration test was performed.

The concentration of organochlorine pesticides in the oyster samples was performed following the technique of Murphy (1972), and modified by Waliszewski et al. (1985). For each season, a sample of 10 g of ground and freeze-dried oyster was weighed out and placed in a Teflon® container with 20 ml of acetone and 20 ml of hexane as solvents. The volume of this solution was divided into two parts, one to be used for the extraction of lipids and the other to determine the concentration of organochlorine pesticides.

The extraction of lipids was performed in 30 min at 110 °C and 200 psi. The extract was collected and placed in 250 ml flasks and left to chill for 30 min, after which 20 ml was removed and the solvent evaporated in a roto-evaporator at a temperature of 45 °C. The extract was then weighed on a digital scale to determine the total lipids in each sample.

For the analysis of organochlorine pesticides, 10 ml of the original solution was placed into a 50 ml tube with a Bakelite plug. Then, 1 ml of sulfuric acid was added and the solution agitated vigorously for 1 min to precipitate the fat, after which the solution was left to rest for 15 min to separate the phases. The supernatant was filtered onto a sodium sulphate layer (8.0 g), washed with 10 ml of petroleum ether and evaporated at 45 °C to a volume of 1 ml of purified sample which was stored in an amber vial (Reacti-vial, Pierce®).

The equipment used for this study was a gas chromatograph (Thermo Electron Model Trace GC Ultra 115V, Thermo Fisher Scientific Inc. ©, Monterrey, Nuevo León, Mexico) with an electron capture detector. The separation of pesticides was performed in a 30m x 0.32mm x 0.25µm chromatographic column with 14% cyanopropylphenyl polysiloxane (Thermo Fisher Scientific Inc©, Bellefonte, Pennsylvania, USA), and a carrier gas of ultrapure nitrogen (Praxair-Mexico) at a flow of 2.5 ml min⁻¹. Operating temperatures were 300 °C for the detector, 250 °C for the injector and 160 to 280 °C for the column (4 °C min⁻¹). The injection volume was 1 µl in splitless mode.

2.3.3 Processing of the Shrimp Samples for Heavy Metal Determination

The material used in the digestion was washed previously with a 10% solution of soap that was neutral and free of phosphates in order to avoid ionic interference in reading the spectrophotometer. Then the material was rinsed with tap water and submerged in a solution of distilled water and 20% nitric acid for 24 h. To assure complete removal of the acid, the material was submerged in type II water for 24 h. Finally, the glassware was drained and dried in a forced air oven (Riossa CF-102) at a temperature of 100 °C for 24 h. For digestion, 0.5 g of each sample was weighed out and placed in a Teflon® container (HP-500) to which 9 ml of 70% nitric acid (reagent grade) was added. This process was carried out at a temperature of 190 °C. Each group of samples was accompanied by one blank sample and a control. At the end of the digestion, the samples were vacuum-filtered into a Nalgene® bottle using 0.45 µm nitrocellulose Millipore® filters. The filtrate was diluted in a flask to a volume of 25 ml with Type II water (obtained with a Milli-Q membrane), after which the diluted samples were transferred to polypropylene flasks and stored at a temperature of 4 °C for subsequent reading. The concentration of Cadmium (Cd) was determined using a Varian SpectraAA – Duo Flame Atomic Absorption Spectrophotometer FS220 (with ultralamps, autodiluter S1PS20 and autosampler SPS5). The concentrations of Lead (Pb) and Arsenic (As) were determined using a Graphite Furnace 220Z, according to NOM-117-SSA1-1994 (Diario Oficial de la Federación, 1995).

2.4 Statistical Analysis

A single-factor analysis of variance was performed on the concentrations of organochlorine pesticides and heavy metals in the oyster, crab and shrimp samples. The pesticide data from oyster samples were transformed using natural logarithms. Tukey tests were performed for the multiple comparisons of means. The data were analyzed using the software Statistica v7.0 (StatSoft, Inc., Tulsa, Oklahoma, USA).

3. Results

3.1 Organochlorine Pesticide Levels in Oysters

Table 1 presents the average concentrations of organochlorine pesticides obtained from samples of oysters, *C. virginica*, collected from the Madre, Mandinga and Mecoacán lagoon systems.

3.2 DDT and Its Metabolites in Crab

Chromatographic analysis revealed the presence of high concentrations of DDT and its metabolites in muscle and viscera samples from *Callinectes rathbunae* during the three sampling seasons (Tables 2, 3).

Table 1 Organochlorine pesticides in oysters *C. virginica* (ng g⁻¹) in the Madre, Mandinga and Mecoacán lagoon systems. (± SD)

COMPOUND	LAGUNA MADRE	LAGUNA MANDINGA	LAGUNA MECOACÁN
HCB (hexachlorobenzene)	287.87 ± 1.43 ^b	203.09 ± 9.29 ^a	163.81 ± 14.26 ^a
Lindane (γ-hexachlorocyclohexane)	25.11 ± 8.39 ^{ab}	147.51 ± 2.09 ^b	1.34 ± 0.00 ^a
Delta-HCH (δ-hexachlorocyclohexane)	38.47 ± 7.88 ^b	1466.65 ± 8.29 ^a	22.46 ± 2.42 ^b
Endosulfan I (alfa)	13.97 ± 0.30 ^a	37.27 ± 0.00 ^a	14.89 ± 1.16 ^a
4-4'-DDE (dichlorodiphenylethane)	22.47 ± 0.00 ^a	44.51 ± 12.45 ^a	n.d. ^b
Dieldrin	355.73 ± 11.83 ^b	159.37 ± 45.00 ^a	45.53 ± 0.00 ^{ab}
Endrin	n.d. ^c	57.43 ± 0.28 ^a	11.89 ± 0.00 ^b
op'-DDT (dichlorodiphenyltrichloroethane)	n.d. ^b	44.43 ± 2.38 ^a	20.30 ± 6.74 ^a
Endrin Aldehyde	n.d. ^b	130.25 ± 6.98 ^a	n.d. ^b
Endosulfan II (beta)	n.d. ^b	99.48 ± 16.21 ^a	n.d. ^b

Note: Values in the same row that have the same letters indicate no significant differences ($p > 0.05$); n.d. = not detected.

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Table 2 Mean concentrations of DDT and its metabolites measured in samples of muscle and viscera from *Callinectes rathbunae* collected during January to October 2008 from Laguna Grande and Laguna Vega de Alatorre, Veracruz, Mexico.

Pesticide (ng g ⁻¹ base lipid)	Windy		Dry		Rainy	
	Muscle	Viscera	Muscle	Viscera	Muscle	Viscera
<i>pp'</i> -DDE	45.81 ± 16.48 ^a (35.62 - 70.39)	38.58 ± 4.58 ^a (32.81 - 43.98)	50.56 ± 48.27 ^a (21.67 - 136.10)	46.28 ± 11.96 ^a (31.99 - 68.24)	26.90 ± 12.19 ^a (17.12 - 44.47)	45.56 ± 15.25 ^a (30.96 - 75.04)
<i>pp'</i> -DDD	33.31 ± 8.00 ^{ab} (27.94 - 45.03)	12.92 ± 17.21 ^b (15.30 - 36.36)	53.70 ± 25.21 ^a (13.43 - 91.76)	15.91 ± 14.65 ^b (19.99 - 39.48)	44.51 ± 7.81 ^a (26.85 - 51.82)	20.96 ± 16.87 ^b (17.28 - 44.44)
<i>pp'</i> -DDT	130.63 ± 30.84 ^{ab} (101.64 - 173.82)	106.87 ± 78.40 ^a (96.45 - 169.68)	115.86 ± 24.82 ^a (89.31 - 155.74)	134.58 ± 70.69 ^{ab} (64.68 - 246.62)	96.54 ± 17.64 ^a (71.71 - 121.84)	216.05 ± 90.91 ^b (112.47 - 291.97)
<i>op'</i> -DDT	57.33 ± 48.72 ^{ac} (48.85 - 118.49)	49.46 ± 46.67 ^{ac} (26.75 - 107.54)	68.82 ± 37.94 ^{ab} (38.13 - 111.71)	27.82 ± 25.60 ^c (34.94 - 69.01)	77.81 ± 13.65 ^a (46.93 - 90.59)	36.65 ± 29.50 ^{bc} (30.21 - 77.69)
Σ-DDT	267.07 ± 63.89 ^{ab} (229.42 - 362.29)	207.82 ± 43.05 ^a (151.51 - 251.01)	288.95 ± 84.70 ^{ab} (163.90 - 407.02)	224.59 ± 54.57 ^a (163.93 - 314.86)	245.76 ± 34.90 ^a (202.08 - 296.47)	319.22 ± 72.19 ^b (230.46 - 459.20)

Note: Values in parentheses indicate the range of concentrations. Values in the same row having the same letters indicate no statistically significant differences ($p > 0.05$)

Table 3 Mean values of the different forms of DDT (ng g⁻¹) and its metabolites found in muscle and viscera samples from *Callinectes rathbunae*

Tissue	<i>pp'</i> -DDE	<i>pp'</i> -DDD	<i>pp'</i> -DDT	<i>op'</i> -DDT	Σ-DDT	<i>pp'</i> -DDT / <i>pp'</i> -DDE
Muscle	41.09	43.84	114.34	67.99	267.26	2.78
Viscera	43.47	16.60	152.50	37.98	250.55	3.51

3.3 Concentrations of Heavy Metals in Shrimp

Mean concentrations of heavy metals in both of the shrimp species sampled are presented in Table 4. Heavy metal concentrations during windy (January – February), and the dry and rainy seasons are presented in Table 5.

Table 4 Concentrations of heavy metals ($\mu\text{g g}^{-1}$) in different species of shrimp from coastal lagoons along the Gulf of Mexico

Especie	Pb	Cd	Cu	Hg
<i>Litopenaeus setiferus</i>	0.8115 ± 0.3420	0.0750 ± 0.0108	n.d.	0.1215 ± 0.0344
<i>Farfantepenaeus aztecus</i>	0.119 ± 0.12	0.032 ± 0.019	18.51 ± 4.109	n.d.

n.d. = not detectable

Table 5 Seasonal variation of heavy metal concentration in shrimp collected from Tamiahua and Alvarado lagoons of Mexico. ($\mu\text{g g}^{-1}$ dry weight)

Lagoon	Species	Season	Pb	Cd	Cu	Hg
Tamiahua	<i>Farfantepenaeus aztecus</i>	Windy	0.0545 ± 0.043	0.033 ± 0.004	16.453 ± 0.033	n.d.
		Dry	0.248 - n.d.	0.042 ± 0.021	19.595 ± 4.915	
		Rainy	n.d.	0.014 ± 0.011	18.439 ± 5.706	
Alvarado	<i>Litopenaeus setiferus</i>	Windy	1.0534 ± 0.1432	0.0674 ± 0.0382	n.d.	0.1459 ± 0.0523
		Dry	0.5698 ± 0.3418	0.0827 ± 0.0290		0.0972 ± 0.0184
		Rainy	n.d.	n.d.		n.d.
			n.d.	n.d.		n.d.

n.d. = not detectable

4. Discussion

4.1 Organochlorine Pesticide Levels in Oysters

All of the pesticides analyzed were found in the Mandinga lagoon system, providing evidence of a contamination problem and risk to public health. In Laguna Madre, the compounds endrin, op' -DDT, endrin aldehyde, and beta-endosulfan were not detected. In the Mecoaacán lagoon system, the compounds pp' -DDE, endrin aldehyde and beta-endosulfan were not detected.

In all the lagoon systems, delta-Hexachlorocyclohexane (delta-HCH) was detected, but the greater concentration was observed in Laguna de Mandinga, with a mean value of $1466.65 \pm 8.29 \text{ ng g}^{-1}$. This can be attributed to the affinity that delta-HCH has for organic matter found in the digestive tract of bivalve molluscs, which is generally composed of 60% detritus, 30% phytoplankton and 10% unidentified material (CNROP, 2004; Muñeton et al., 2001). Castañeda et al. (2007) report that α , β and γ -HCH have been detected during these three seasons of the year in *C. virginica* from the lagoon systems of La Mancha and Alvarado, in the state of Veracruz, Mexico, along the Gulf of Mexico. The presence of such compounds was assumed to be associated with the predominance of banana cultivation. Other authors associate the presence of these compounds with the cultivation of corn, beans, mangos, pineapple, chiles, lime, grasses, red and green tomatoes, maradol papayas and watermelon, all of which are characteristic crops of zones adjoined to the lagoon systems studied

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(Table 6). According to Randall et al. (1998), the presence of γ -HCH coincides with the application of the pesticide lindane, which is utilized for the control of cattle ectoparasites.

Table 6 Principle developmental production activities within the study area

Lagoon	Economic activities	Primary crops	References
Madre	Agriculture Ranching (cows and goats) Mining (zinc, dolomite, copper and silver)	Sorghum, com, cotton, beans, watermelon, <i>Agave</i> , safflower, citrus, soybeans and recently aloe vera	Bello et al, 2009; Benítez & Bárcenas, 1996
Mandinga	Agriculture Ranching (cattle) Gas and basic petrochemicals	Corn, beans, mangos, pineapple, chiles, limes, grasses, red and green tomatoes, Maradol papayas, watermelon	Zorrilla, 2006
Mecoacán	Agriculture Ranching (cattle, pigs and birds)	Cultivated pastures, coconuts, cacao, pepper, plantains, com, beans, sugarcane, yucca and mangos	INEGI, 2012

Another pesticide that was present at medium-high concentrations was hexachlorobenzene (HCB) which was detected in *C. virginica* from Laguna Madre ($287.87 \pm 1.43 \text{ ng g}^{-1}$). These compounds are characteristically deposited in the sediments of lagoon systems, and the sedimentation process is enhanced by the stability of the water column which favors the association of this compound with benthic organisms, as is the case with the oyster *C. virginica*. In this way, the particles in suspension, upon being filtered by this organism, are incorporated into the tissues and allow these compounds to bioaccumulate (ATSDR, 2002).

Dieldrin is a persistent organic pesticide whose use has been restricted since 1970 in the United States and countries included in the Stockholm Convention of 2001 (COP, 2001). However, in 1972 the Environmental Protection Agency of the United States (EPA) approved the use of aldrin and dieldrin for the control of termites (ATSDR, 2002b). In 1980 the use of these compounds was prohibited again due to their carcinogenic effects (TREHI, 2001). Nevertheless, in this study dieldrin was found in the lagoon systems of Madre, Mandinga and Mecoacán, with concentrations of 355.73 ± 11.83 , 159.37 ± 45.00 , and $45.53 \pm 0.00 \text{ ng g}^{-1}$. The presence of dieldrin is due to the degradation of aldrin that is applied to corn and potatoes for pest control (WHO, 2003; ELIKA, 2008), as well as to the capacity that dieldrin has to adhere to sediments in aquatic systems and remain stable (ATSDR, 2002b).

Endrin is a pesticide used since the 1950s to counteract diverse agricultural pests, particularly in rice cultivation, although it is also used on sugarcane and corn, and is also employed as a rodenticide (EPA, 2012). This compound is adsorbed in water and sediments, has a half-life of 10 years, accumulates in the tissues of aquatic organisms and can be toxic (ATSDR, 1997). At concentrations of 100 ng g^{-1} it can reduce shell growth in mature oysters by 51% (Wilber, 1971). The FDA (2002), established a maximum permissible limit of 0.30 ppm ($\mu\text{g g}^{-1}$) for the consumption of bivalve molluscs. The presence of this pesticide in the lagoon systems in the states of Tabasco and Veracruz is due to persistent use of this compound in agricultural activities (Díaz & Rueda, 1996). However, it was not detected in samples of *C. virginica* from Laguna Madre. The pesticide endrin aldehyde was not detected in Laguna Madre or Laguna de Mecoacán, and was only found in samples from Laguna de Mandinga with an average concentration of $130.25 \pm 6.98 \text{ ng g}^{-1}$. This is because the percentage of endrin that is degraded to endrin aldehyde is low due to the

chemical characteristics of endrin which require that it be exposed to high temperatures and prolonged periods of light to transform it into endrin aldehyde. Such conditions are not found in the lagoon systems where the average depth is 1.5 m, the temperature is relatively low and turbidity is high, which impedes the penetration of light (ATSDR, 1997).

Endosulfan is another pesticide widely used in Mexico and is considered important for study because of its high toxicity in aquatic organisms and risk to public health (INE, 2011). This pesticide has two isomers, endosulfan I and II. In this study, endosulfan I had the greatest mean concentration in samples from Laguna de Mandinga ($37.27 \pm 0.00 \text{ ng g}^{-1}$) and the smallest from Laguna Madre ($13.97 \pm 0.30 \text{ ng g}^{-1}$). Based on these concentrations, Laguna de Mandinga has a value 50% below the maximum concentration permitted (74 ng g^{-1}) that was established by the EPA for water and aquatic organisms, while Laguna Madre has a value 71% under the limit (EPA, 2000). Although endosulfan is dispersed in the air, water and sediments, it can be degraded in weeks, but it can also remain for years adhered to soil particles without being degraded. The properties of this compound are worthy of noting, due to the sedentary habits of benthic organisms like oysters that are chronically exposed to this pesticide (INE, 2011). Indeed, some ecotoxicological effects in oysters are referred to as genotoxic and embryotoxic (UNEP, 2009).

Endosulfan II had a mean concentration of $99.48 \pm 16.21 \text{ ng g}^{-1}$ in samples from Laguna de Mandinga, while in the lagoon systems of Madre and Mecoacán it was not detected. Benthic organisms ingest and easily incorporate this pesticide because of its solubility in water, low persistence, and easy elimination (UNEP, 2009). The concentration reported in this study showed that benthic organisms are exposed continuously to this insecticide because of its use in the cultivation of tomatoes, corn and coffee.

The DDT isomers included in this study were *op'*-DDT and *pp'*-DDE. Laguna de Mandinga had the highest mean concentrations (44.43 ± 2.38 and $44.51 \pm 12.45 \text{ ng g}^{-1}$, respectively), values within the ranges detected for the lagoon systems of La Mancha and Alvarado (Castañeda et al., 2011). DDT is used in the control of malaria vectors and its presence in the lagoon systems is due to the overland runoff that transports sediments into the aquatic systems where they remain available to benthic organisms. DDT and its isomers DDE and DDD can remain in sediments for long periods of time, possibly hundreds of years (ATSDR, 2002c).

Its extensive use in subtropical and tropical areas of the Gulf of Mexico has promoted the bioaccumulation and biomagnification of DDT and its metabolites, as well as the incorporation of these compounds throughout the trophic web, resulting in problems for public health (Castañeda et al., 2011).

4.2 Organochlorine Pesticides in Crab

Among benthic organisms, the crab *C. rathbunae* is the most important fishery resource in Laguna Grande in Vega de Alatorre. Castañeda et al. (2011) estimated the concentrations of DDT and its metabolites in this system, and found relatively high concentrations as a consequence of their use in sanitary campaigns to control dengue. It is for this primary reason that studies on this organism have focused on detecting the presence of DDT.

According to the results obtained in this study, the predominant compound was *pp'*-DDT with a mean value of $136.35 \pm 70.00 \text{ ng g}^{-1}$, followed in descending order by *op'*-DDT (52.90 ± 35.80), *pp'*-DDE (42.30 ± 24.61) and *pp'*-DDD ($31.64 \pm 22.06 \text{ ng g}^{-1}$). *pp'*-DDE was the only metabolite that did not show statistically significant differences ($p > 0.05$) in the mean concentrations from viscera and muscle tissue samples, within or among seasons. The minimum and maximum

concentrations of this compound were found in muscle and varied between 26.90 and 50.56 ng g⁻¹ (Table 2). *pp'*-DDD and *op'*-DDT showed similar patterns of bioaccumulation in both tissues, but with significantly greater concentrations in muscle ($p < 0.05$) during the dry and rainy seasons (Table 2).

On the contrary, *pp'*-DDT had a greater concentration in samples of crab viscera than in muscle tissue, and showed significant differences ($p < 0.05$) during the rainy season where the concentrations varied from 96.54 in muscle to 216.05 ng g⁻¹ in viscera. In terms of total DDT value (the sum of *pp'*-DDE, *pp'*-DDD, *pp'*-DDT and *op'*-DDT), a decline in accumulated concentrations in the muscle was observed in samples collected during the rainy season, and differed significantly ($p < 0.05$) with the values detected in the viscera samples (Table 2). The greatest concentration of total DDT was measured in viscera samples collected during the rainy season (319.22 ng g⁻¹) and the lowest value was registered in samples of the same tissue during the windy season (207.82 ng g⁻¹).

Table 3 shows the values of the ratio DDT / DDE for muscle and viscera tissue samples from *C. rathbunae*. The relation was 2.78 for muscle and 3.51 for viscera due to the biomagnification of the concentrations of *pp'*-DDT. Despite these values, there were no significant differences ($p > 0.05$) in the levels of DDT accumulated in both tissues.

It is probable that the greater quantity of DDT in this ecosystem stemmed from the overland runoff into the Cerrito, Diamante and Carey rivers (Fig 1) during the rainy season. The process was magnified by the strong winds during the windy season that transported pesticides from their application sites to the lagoon. The organic matter from these rivers acts intermediately to the processes of deposition and circulation in the lagoon, permitting the DDT to remain suspended in the body of water by attaching to particles or becoming trapped primarily in the superficial layers of sediment due to its low solubility and high persistence in these environments (ATSDR, 1994; Willman et al., 1997). The high levels of DDT found in *C. rathbunae* can be explained by their feeding habits; they are considered benthic opportunistic omnivores (Perry & McIlwain, 1986), whose diet consists preferably of bivalve molluscs, fish, and deposited and suspended organic matter (detritus, phytoplankton and zooplankton) (Guillory et al., 2001; Gorni & Weber, 2004; Gómez, 2009). This also results in biomagnification because this pesticide passes through one or more levels of the trophic web (Clark et al., 1988; Morales & Cobos-Gasca, 2001).

4.3 Heavy Metals in Shrimp

The Gulf of Mexico basin contains excessive amounts of some metals in its coastal ecosystems due to their introduction as river contaminants. Nearly 95% of the metals transported by the rivers are deposited along the coastal margins including estuaries, the continental shelf and the continental slope (Botello et al., 2004). Benthic organisms are the most affected by heavy metals in ocean and coastal waters, due to their direct interaction with sediments (Laws, 2000).

There is greater heavy metal contamination along the coastal zones of the Gulf of Mexico, particularly from Pb, Cd and Cr (Botello et al., 2004). These metals can produce adverse toxicological effects in benthic organisms inhabiting coastal zones, and negatively affect coastal lagoon systems such as Tamiahua and Alvarado.

The concentration ranking of heavy metals in the shrimp *F. aztecus* from the Tamiahua lagoon system was Cu > Cd > Pb, while for *L. setiferus* from the Alvarado lagoon system the concentration ranking was Pb > Cd > Hg. The concentrations of Pb and Cd from *F. aztecus* complied with the specifications of NOM-242-SSA1-2009 (Diario Oficial de la Federación, 2011), and were lower than 0.5 µg g⁻¹ for both metals. Copper had maximum concentrations although its maximum

permissible limits are not found in Mexican legislation, nor for the European Community, although the United Kingdom has established a permissible limit of $20.0 \mu\text{g g}^{-1}$ (UK, 1998), and the FDA (1993) of $32.5 \mu\text{g g}^{-1}$.

In *F. aztecus* and *L. setiferus* the maximum levels of Cd were registered during the dry season, as was that for Cu in *F. aztecus* and for Pb in *F. aztecus* during winter. In *F. aztecus* Cu had greater concentrations than Cd and Pb in the seasons studied. Significant differences were observed for the concentrations of Cu, Cd and Pb over seasons in the sampling sites ($p < 0.05$).

No significant difference existed ($p > 0.05$) for *L. setiferus* among seasons for the concentrations of Pb, Cd and Hg, but differences were observed between the levels of Pb and those obtained for other metals ($p < 0.05$). Pb and Hg had their highest concentrations during winter, 1.0534 ± 0.1432 and 0.1459 ± 0.0523 , respectively. In contrast, Cd had a minimal concentration during this season ($0.0674 \pm 0.0382 \mu\text{g g}^{-1}$).

Phillips & Rainbow (1989) associated the high concentrations of Cu in shrimp muscle and other tissues to its function as an essential element for their development. In decapod crustaceans its concentration is regulated as a function of their metabolic needs, despite the high concentration in the environment. Palomarez et al. (2009) also related differences in the concentrations of this metal to species, geographical zone, biological factors such as the total size of the organisms analyzed and the present concentrations in the environment. Likewise, Mendoza (2010) obtained a similar maximum concentration of $18.625 \mu\text{g g}^{-1}$ in samples of *F. aztecus* from Laguna de Tampamachoco.

The maximum concentration of Pb was in samples of *L. setiferus* from the Alvarado lagoon system, while concentrations of this metal in samples of *F. aztecus* from the Tamiahua lagoon system were much lower. According to Viarengo (1993), the presence of high concentrations of Pb in shrimp is related to its competition with Cu, which is a bioessential element in tissue ionic regulation processes.

The concentration of Cd in *F. aztecus*, as in *L. setiferus*, was elevated in samples from both lagoon systems, ranking it third in terms of concentration. However, the levels were low in both cases and were within the permissible limits set by Mexican norms, as well as being below the limits set for the consumption of aquatic food ($0.20 \mu\text{g g}^{-1}$ Cd) (Nauen, 1983; Lango et al., 2010).

Because Hg is a nonessential metal, it is highly toxic for all organisms (Wallace et al., 1982; Landis & Yu, 1992). As such, body concentrations of nonessential metals such as Hg or Cd cannot be regulated by some crustaceans (Frías et al., 2001), resulting in high accumulated levels in tissues and occasional organism death (Zanders & Rojas, 1992; Frías et al., 2001).

The concentrations of Hg obtained for the lagoon systems during the 3 seasons studied were below the limits stipulated by NOM-242-SSA1-2009 that report the concentration of mercury as methylmercury ($0.5 \mu\text{g g}^{-1}$), a limit that establishes a different concentration than that reported by Nauen (1983) of $2.5 \mu\text{g g}^{-1}$ dry weight, and the FDA (1978) with $5.0 \mu\text{g g}^{-1}$ dry weight. The low levels registered in this investigation (0.1459 ± 0.0523 , 0.0972 ± 0.0184), and those not detected (n.d.) during the winter, dry and rainy seasons, were similar to those reported by Reymer & Reymer (1975) of a mean of $0.06 \pm 0.12 \mu\text{g g}^{-1}$ (wet weight) in samples of *F. aztecus* from Laguna de Tampico, $0.04 \pm 0.02 \mu\text{g g}^{-1}$ and $0.03 \pm 0.04 \mu\text{g g}^{-1}$ in samples of *L. setiferus* from the Port of Veracruz and Laguna de Términos, respectively. As well, Báez et al. (1975) recorded a range of $0.16\text{-}0.85 \mu\text{g g}^{-1}$ for the same species from the Coatzacoalcos River.

5. Conclusions

The levels of pesticides in oysters and crabs indicates a constant contribution of these compounds which are used in livestock operations, agricultural activity and the sanitary campaigns against dengue and malaria vectors that are performed in zones adjoined to the lagoon systems studied. Laguna de Mandinga had the greatest concentrations of pesticides in comparison with Laguna Madre and Laguna de Mecoacán. The bioaccumulation of DDT and its metabolites in the muscle and viscera of *C. rathbunae* were similar in both tissues. *pp'*-DDT was the most dominant of the metabolites, indicating recent contributions of this compound into the lagoons as a consequence of the sanitary campaigns to control human disease vectors. The levels of DDT found in the samples of muscle and viscera from *C. rathbunae* exceeded the permissible maximum limits established by the FDA (1984) for aquatic organisms destined for human consumption, and those of the Norma Oficial Mexicana (NOM-031-SSA1-1993) which states that fresh or refrigerated molluscs should not contain DDT or residuals of prohibited pesticides (Diario Oficial de la Federación, 1995) listed in the catalogue of pesticides.

The persistent metals in the shrimp *F. aztecus* and *L. setiferus* were Cd and Pb, and their concentrations in *F. aztecus* complied with the specifications of NOM-242-SSA1-2009 (Diario Oficial de la Federación, 2011) and were lower than 0.5 µg g⁻¹ for both metals. Normative legislation is not available for Hg and Cu.

The concentrations of pesticides and metals in benthic organisms of commercial importance are related to the habitat and feeding habits (detritivores and filterers) of these animals. As such, the consumption of these compounds permits their bioaccumulation in the tissues of these organisms and in those of human consumers, thus constituting a public health risk. This situation directly affects consumers living along the Gulf of Mexico and anywhere these products are marketed.

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