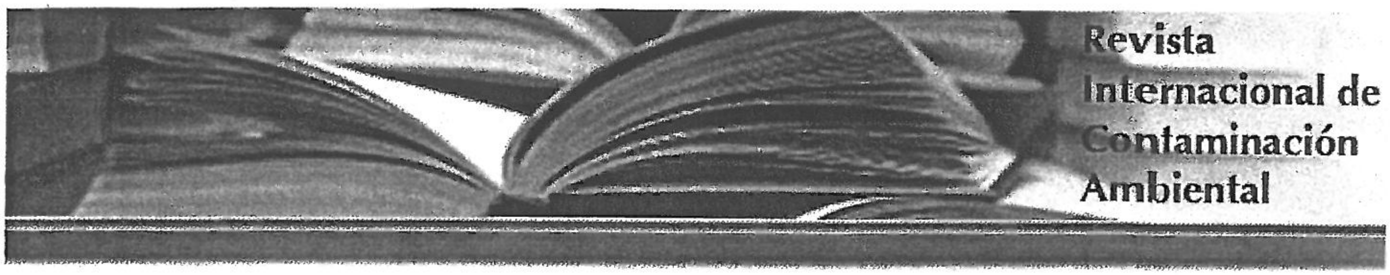

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En esta revista se aceptan para su publicación trabajos originales y de revisión sobre aspectos físicos y químicos de la contaminación, investigaciones sobre la distribución y los efectos biológicos y ecológicos de los contaminantes; así como sobre tecnología e implementación de nuevas técnicas para su medida y control; también son aceptados estudios sociológicos, económicos y legales acerca del tema. Se publicarán los escritos que mediante arbitraje de especialistas y a juicio del Consejo Editorial tengan el nivel y la calidad adecuados para ello y su contenido será responsabilidad única de los autores.

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BIOACCUMULATION OF CADMIUM (CD), LEAD (PB) AND ARSENIC (AS) IN *Crassostrea Virginica* (GMELIN, 1791), FROM TAMIAHUA LAGOON SYSTEM, VERACRUZ, MÉXICO

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Key words: oyster, contamination, bivalve, gonad-digestive gland, muscle-mantle-gill

ABSTRACT

The accumulation of heavy metals in oysters, *C. virginica*, from Tamiahua Lagoon System along the gulf coast in the state of Veracruz, México, results from inputs provided by anthropogenic activities and the physicochemical and ecophysiological processes occurring in these systems. The objective of this study was to determine concentrations of Cd, Pb and As in gonad-digestive gland (GDG) and muscle-mantle-gill (MMG) tissues in females and males of *C. virginica* from Tamiahua Lagoon. Two sampling sites were selected, and each sample consisted of 500 oysters of commercial size. Concentrations of Cd, Pb and As were determined using atomic absorption spectrophotometry and a graphite furnace. The highest concentrations were found in MMG tissues, whose mean values for these metals are 11.77 ± 1.32, 0.484 ± 0.08, 4.02 ± 0.56 mg kg^{-1}. Cadmium concentrations exceeded the limits for the consumption of bivalve mollusks established by the sanitary regulations, indicating a risk to human health.

Palabras clave: ostión, contaminación, bivalvo, gónada-glándula digestiva, músculo-manto-branquia

RESUMEN

La acumulación de metales pesados en ostión *C. virginica* de las lagunas del Golfo de de México, se debe principalmente a las descargas de actividades antropópicas y a procesos fisicoquímicos y ecofisiológicos que ocurren en estos sistemas. El objetivo de este estudio fue determinar las concentraciones de Cd, Pb y As en las porciones gónada-sistema digestivo (GSD) y músculo-manto-branquias (MMB) de hembras y machos de ostión en el sistema lagunar de Tamiahua, Veracruz. Se seleccionaron dos sitios de muestreo. Cada muestra consistió de 500 organismos de talla comercial. Las concentraciones de Cd, Pb y As se determinaron por espectrofotometría de absorción atómica y horno de grafito; el tejido MMB registró las concentraciones más altas, de estos metales con valores medios de 11.77 ± 1.32, 0.484 ± 0.08, 4.02 ± 0.56 mg kg^{-1}. El Cd superó los límites permisibles de consumo que establecen las normas sanitarias para moluscos bivalvos y se estima que representa un riesgo para la salud humana.

INTRODUCTION

A large variety of native mollusks exist in México, of which many species are found in the Gulf of México. One of these, the American oyster *Crassostrea virginica* is highly important because it is the sixth species most produced at the national level with 51 339 tons/year. Nationally, the state of Veracruz is the main producer of this oyster species along the gulf coast, with 24 475 tons harvested in 2003 (SAGARPA 2006). However, the production of this species in the state over the last several years has been affected by biological (bacterial and viral) and chemical (heavy metals, organochlorate pesticides, and hydrocarbons) contamination and pollution (Barrera-Escorcia and Wong-Chang 2005, Albert and Benítez 2005, Guzmán-Amaya *et al.* 2005, Páez-Osuna 2005, Robert *et al.* 2008). Above thresholds of acceptance, these contaminants are considered pollutants, causing problems with food safety and health of the oysters produced (Munro and Chaibonneau 1981, Rainbow 1990, Páez-Osuna 1996, Galaviz 2003, SENASICA 2003, Castañeda-Chávez *et al.* 2005).

Permissible limits of heavy metals in oysters for human consumption in the European Union, United States of America, Spain, and México are listed in **table I**. These countries regulate overall oyster production including cultivation, wild harvesting, and marketing, and legislations exist dictating whether or not oysters can be cultivated or harvested from certified areas in order to ensure oyster quality and to avoid public health problems (SENASICA 2003). However, oyster harvesting and culture in México, and particularly in the Gulf region, is carried out in uncertified areas which are exposed to anthropogenic contamination and pollution (Wong-Chang and Barrera-Escorcia 2005).

Although in México there are few studies on heavy metal pollution of bivalve molluscs in comparison with other countries, some have reported physiological alterations in these animals. Gold *et al.* (1995) reported damage to the gills, digestive tract, and connective tissue in the diverticulum in *C. virginica* collected from the lagoons of Mecoacan, Carmen and Machona in the state of Tabasco, from Cd, oil, and associated environmental interactions. Additional studies conducted in the lagoons of Sontecomapan, La Mancha, Alvarado, Mandinga and Tamiahua in the state of Veracruz have reported the concentrations of heavy metals in *C. virginica* without examining the physiological effects on these animals or their impact on public health (Luna e*t al.* 2002, Aguilar and Amador del Ángel 2003, Lango *et al.* 2003, Guzmán-Amaya *et al.* 2005, Baqueiro-Cárdenas *et al.* 2007a, b).

TABLE I. AVERAGE CONCENTRATIONS OF HEAVY METALS (mg kg^{-1}) OBSERVED IN OYSTERS, *C. virginica*, SAMPLED FROM TAMIAHUA LAGOON, VERACRUZ, MÉXICO, AND THE CONCENTRATIONS FOR OYSTER CONSUMPTION PERMITTED BY OTHER COUNTRIES

Metal	Permissible Limits					
	Oyster		Oyster			
	a	b	México	USA	EEC	Spain
Cd	7.32	21.41	0.5	4.0	2.0	1.0
Pb	21.42	0.78	1.0	1.7	3.0	3.0; 1.5[2]
As	-	7.36	2 to 4[1]	86.0	n.s.	4.0
Hg	-	-	1.0	1.0	1.0	0.5
Cr	33.64	-	n.s.	13.0	n.s.	-
Ni	7.62	-	n.s.	80.0	n.s.	-
Cu	202.43	-	-	-	-	20
Zn	156.85	-	-	-	-	-
Al	-	-	-	-	-	-
Co	39.73	-	-	-	-	-
Sn						250

a. concentrations reported by Anton and Lizaso (2002), Galaviz (2003), and Guzmán-Amaya *et al.* (2005)
b. concentrations reported in this investigation
Mexico: NOM-031-SSA1-1993
USA: (FDA, 1993a, b, c, d, e, f)
EEC: European Economic Community (Codex Alimentarius, 1995)
Spain: Norma de Calidad para Moluscos Bivalvos Depurados, 1985 (FAO, 1989)
n.s. = no standards
- = no registration
[1] maximum permissible limit NOM-129-SSA1-1995
[2] DOUE, 2006

Bioaccumulation and biomagnification of heavy metals in oysters have implications for human health because they are a popular dietary item. The risks that these metals present to public health include: 1) Cd affects digestion, bones, reproduction, the central nervous and immune systems, and causes brain damage and cancer; 2) Pb can damage the brain and kidneys, interfere with the synthesis of hemoglobin, alter gastrointestinal function, harm the reproductive system, and cause acute and chronic damage to the nervous system; 3) As is deposited in the hair and nails, and can cause cancer in the skin, kidneys, bladder, scrotum, liver, lymph system, and lungs (Anton and Lizaso 2002, ATSDR 2005, 2007). The objective of this study was to determine concentrations of Cd, Pb, and As in the gonad-digestive gland (GDG) and the muscle-mantle-gill (MMG) tissues of females and males of *C. virginica* from Tamiahua Lagoon in the state of Veracruz, México.

MATERIALS AND METHODS

Study area and sampling method

The Tamiahua Lagoon is located along the gulf coast of México in the northern part of the state of Veracruz. It is bordered by the municipalities of Tamiahua, Ozuluama, and Tampico (between 21° 06' to 21° 20' N, and 97° 23' to 97° 46' W), and covers an area of 88 000 ha (**Fig. 1**).

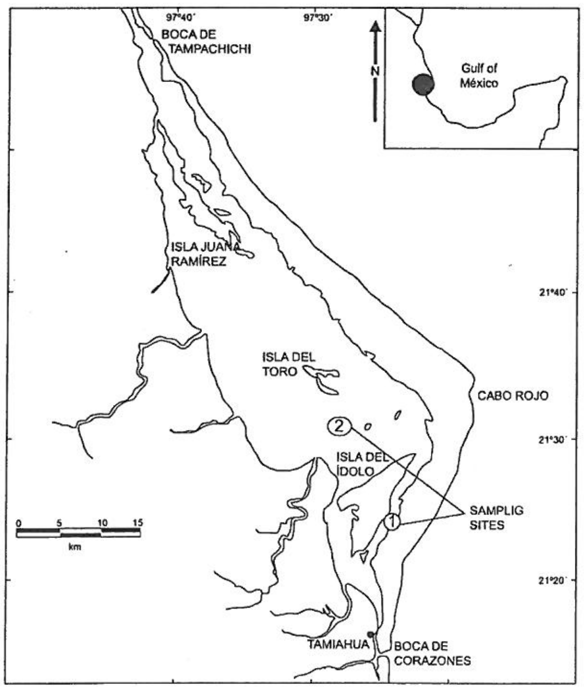

Fig. 1. Sampling site locations (1 and 2) in Tamiahua Lagoon, Veracruz, México

Two sites were selected for oyster sampling, site 1 in the central part of the lagoon, and site 2 in the southern portion (**Fig. 1**). Samples were collected once per month during January, March, April, May, June, and July 2004. Physicochemical variables were measured in situ using an aquatic probe (YSI Model 6600 multiparameter data logger - Yellow Springs, OH 45387-1107, USA), and included salinity, temperature, and pH.

Six samples were collected from each site, for a total of 12 samples collected each month. Each sample consisted of 500 oysters of harvestable size (10 ± 3 cm), which were thoroughly cleaned to remove adhering debris and algae, and then transported on ice in thermal containers according to the protocols of NOM-109-SSA1-1994 (SSA, 1994). The Condition Index (CI) (Lucas and Beninger 1985) was determined for each sample using 30 oysters from each sample. The sex ratio of each sample was determined using microscopic analyses of gonad smears, with each determination graded into four categories: male, female, hermaphrodite, or undifferentiated (Lango-Reynoso *et al.* 1999, 2000).

Determination of heavy metal concentrations in samples

Thirty males and 30 females were selected from every sample, and from every oyster the GDG and MMG tissues were removed and frozen at –87 °C in an ultrafreezer. The samples were dehydrated in a lyophilizer (ThermoSavant, Model OD-114, Holbrook, NY) for 72 hours at –49 °C and 36x10^{-3} mbar. After dehydration, the samples were milled and screened using No. 30 mesh with an opening of 595 µm, and the ground material stored in Ziplock® bags. The samples of GDG and MMG tissues were digested in a microwave oven CEM Model MARS 5 (CEM Corporation, Matthews, NC) following the Oyster Pure method (EPA 1996). Upon completion of digestion, the samples were filtered into Kitazato flasks using a Fluoropore™ membrane and a vacuum pump, and diluted to volume of 25 mL in a volumetric flask using Type II water. The diluted samples were transferred to polypropylene jars for later analysis. Every sample of tissue was analyzed using two replicates. Concentrations of Cd were determined using a Duo Varian Spectra atomic absorption spectrophotometer with FS220 Flame (containing ultra lamps) and SIPS20 Autodilutor, and an SPS5 Autosampler (Mulgrave, Victoria, Australia). Concentrations of Pb and As were determined using a 220Z graphite furnace (NOM-117-SSA1-1994) (SSA 1994). In order to assure the precision of lab results, use was made of reference standards of NIST Oyster Tissue 1566a.

Statistical analysis

Heavy metal concentrations from the GDG and MMG oyster tissues from the two sites were examined for normality using the Wilks-Shapiro test. A Manova (Statistica version 7.0) was subsequently used to assess significant effects from month, sex, and tissue, and the interactions of month-sex, sex-tissue, and month-sex-tissue.

RESULTS

Average concentrations of Cd, Pb, and As by sampling site, tissue type (GDG or MMG), and sex in *C. virginica* from Tamiahua Lagoon are presented

TABLE II. AVERAGE CONCENTRATIONS (mg kg^{-1}) OF Cd, Pb AND As IN GONAD-DIGESTIVE GLAND (GDG) AND MUSCLE-MANTLE-GILL (MMG) TISSUES IN FEMALES AND MALES OF THE AMERICAN OYSTER, *C. virginica*, FROM TAMIAHUA LAGOON, VERACRUZ, MÉXICO

Metals	Sample site 1				Sample site 2			
	Females		Males		Females		Males	
	GDG	MMG	GDG	MMG	GDG	MMG	GDG	MMG
Cd	9.75 ± 3.26	11.43 ± 2.07	8.03 ± 2.19	12.00 ± 3.09	9.54 ± 4.44	10.35 ± 1.76	11.01 ± 4.05	13.54 ± 1.96
Pb	0.23 ± 0.09	0.56 ± 0.11	0.28 ± 0.18	0.42 ± 0.25	0.21 ± 0.13	0.42 ± 0.19	0.43 ± 0.17	0.56 ± 0.30
As	3.08 ± 0.53	4.64 ± 0.75	2.60 ± 0.65	3.32 ± 0.54	3.78 ± 1.23	4.30 ± 0.82	3.77 ± 0.78	3.96 ± 0.57

in **table II**. Manova results ($P < 0.05$) between sampling sites, sex, months, and tissue for *C. virginica* during the period from January to July, 2004, are presented in **table III**. Tukey test results for multiple comparisons of average concentrations of Cd for month by tissue interaction are shown in **table IV**. Average concentrations by site, based on dry weight for Cd, Pb, and As are compared with the permissible limits provided by the Norma Mexicana NOM-031-SSA1-1993, and similar limits for the United States of America, the European Economic Community, and Spain in **table I**.

DISCUSSION

Cadmium (Cd)

Cadmium was the predominant heavy metal in the GDG and MMG tissues of *C. virginica*, with an average concentration of 21.42 mg kg^{-1} (**Table I**). Botello (1994) reported an average concentration of 1.10 mg kg^{-1} of Cd from the lagoons of Llano, Veracruz, Galaviz (2003) reported an average concentration of 5.86 mg kg^{-1} of Cd from the lagoon at La Mancha, and Guzmán-Amaya *et al.* (2005) reported average concentrations of 2.94 and 4.61 mg kg^{-1} of Cd from the lagoons at Mandinga and Alvarado. Concentrations of Cd found in the coastal lagoons mentioned above, including those observed in this study, are mainly due to the contributions by runoff (Guzmán-Amaya *et al.* 2005). In comparison to the aforementioned lagoons, average Cd concentrations from Tamiahua lagoon were higher. Likely causes for these concentrations include: a) the discharge of untreated domestic and municipal sewage effluent containing organic material, pesticides, fertilizers, detergents, and metals (Cuevas *et al.* 2006); and b) the high level of agricultural activity in the areas surrounding the lagoon (the estimated spatial area for agricultural activity is 68 311.154 hectares, of which 46 645.851 hectares are integrated into 3266 production units) that practice monoculture which requires large quantities of agrochemicals (UNEP 2000, Altieri and Nicholls 2007), particularly the use of phosphate fertilizers containing Cd that are commonly applied to corn, beans, green chilies

TABLE III. MANOVA RESULTS FOR THE CONCENTRATIONS OF Cd, Pb AND As BY SAMPLING SITE, SEX, MONTH AND TISSUE FOR *C. virginica* FROM JANUARY TO JULY 2004, FROM THE TAMIAHUA LAGOON, VERACRUZ, MEXICO

Source of variation	Cd					Pb				As			
	DF	SS	MS	F	P	SS	MS	F	P	SS	MS	F	P
Sites	1	7.81	7.81	0.75	0.38	0.02	0.02	0.55	0.45	3.54	3.54	4.24	***0.04**
Month	5	196.84	39.37	8.24	***0.00**	0.42	0.08	3.48	***0.01**	11.39	2.27	3.90	***0.01**
Sex	1	9.22	9.22	1.93	0.17	0.03	0.03	1.58	0.22	3.47	3.47	5.96	***0.02**
Tissue	1	60.54	60.54	12.67	***0.00**	0.20	0.20	8.36	***0.00**	6.72	6.72	11.53	*0.00
Month by sex	5	7.09	1.42	0.29	0.91	0.29	0.05	2.36	0.07	0.27	0.05	0.09	0.99
Month by tissue	5	74.30	14.86	3.11	***0.02**	0.18	0.03	1.47	0.23	1.78	0.35	0.61	0.69
Sex by tissue	1	12.06	12.06	2.52	0.12	0.23	0.23	9.55	0.06	1.02	1.02	1.76	0.19
Month by sex by tissue	5	6.42	1.28	0.26	0.92	0.27	0.05	2.26	0.08	3.26	0.65	1.12	0.37

DF=degrees of freedom, SS=sum of squares, MS=mean square, F=F-value
P=probability based on α=0.05, significant values in bold.

TABLE IV. AVERAGE CONCENTRATIONS OF Cd (mg kg^{-1}) FOR THE INTERACTION OF MONTH BY TISSUE IN SAMPLES OF *C. virginica* DURING THE PERIOD FROM JANUARY TO JULY 2004, FROM THE TAMIAHUA LAGOON, VERACRUZ, MÉXICO

Month by Tissue	January	March	April	May	June	July
GDG	5.08^{a}	6.49^{ab}	9.14^{abc}	12.35^{cd}	13.15^{cde}	11.29^{bcde}
MMG	9.28^{a}	11.36^{a}	14.22^{a}	12.79^{a}	12.49^{a}	10.84^{a}

Values with different superscript are statistically different, $P<0.05$

and orange crops in the region and eventually leach from the soil and are transported to the lagoons with runoff; c) the ingestion and accumulation of Cd in molluscs is a function of exposure conditions, physiological factors, reproduction, and excretion; factors that also are variable. Hence, interpretations of the mechanisms can be complicated by these and other factors involved during exposure and toxicokinesis (Rodríguez de la Rua *et al.* 2005).

Due to the processes described above, the concentration of Cd in water in coastal Mexican lagoons exceeds the maximum permissible limit (MPL) set forth in the NOM-001-ECOL-1996 (SEMARNAT 1996), which sets the maximum allowable limits for contaminants in wastewater discharges into water and national assets. In this and similar lagoons, which generally have brackish water, the bioavailability of cadmium is the result of continual changes of salinity which determines the concentration of this metal in sessile animals such as *C. virginica* (Márquez *et al.* 2000). In the present study, the concentrations of Cd in the oyster samples were relatively high. In comparison to other studies, the MMG tissues in the present study had higher average concentrations of Cd in both sampling sites, and in females and males, with values of 9.28 to 14.22 mg kg^{-1} respectively. Further, concentrations of Cd were higher in MMG tissues of males, and the values fluctuated between 10.35 and 13.54 mg kg^{-1}. Walsh and O'Halloran (1998) reported that heavy metal accumulation could be ranked by different tissues beginning with gills, kidneys, digestive gland, mantle, and adductor muscle, and that there was a high correlation between some tissues such as digestive gland-kidney and gills-adductor. The process of incorporation of Cd into the MMG tissues of *C. virginica* that were analyzed in this study most likely followed the primary routes of incorporation of metals into aquatic invertebrates, in which water and food were the main transport vehicle (Badii and Garza 2005, Guzmán-García *et al.* 2007).

Regarding the average concentrations of Cd observed in *C. virginica* during January to July 2004, the GDG tissues presented the lowest concentration, with a value of 13.15 mg kg^{-1} in June, while the highest concentration for MMG tissues was 14.22 mg kg^{-1} during the month of April (**Table IV**). The Manova analysis showed significant differences in the concentrations of Cd for the months studied (**Table III**). In the interaction between month and tissue (**Table IV**), concentrations of Cd in MMG tissues were higher than those for GDG tissues during the months January to May. However, in the months of June and July, the concentrations of Cd in GDG tissues were higher than those for MMG. The variation in Cd concentration in the GDG tissues coincides with the breeding season of *C. virginica*, which takes place from May to July, and corresponds precisely with the period of gametogenesis in this species (Lango-Reynoso *et al.* 1999, 2000, Arias 2006, Baqueiro *et al.* 2007b). The concentration of Cd in these tissues also tends to increase during the dry season, due to the sedimentation of particles containing Cd from the water column and the greater exposure time of the oysters to these particles (Palomarez-García *et al.* 2007, 2009). Concentrations of Cd may be related in the MMG tissues the interaction of month by tissue, due to the amount of water involved in food uptake and waste disposal. In addition, these tissues have direct contact with the environment, and both water and food are the principle routes of entry for heavy metals. Consequently, the MMG tissues accumulate more heavy metals than the GDG tissues. In other bivalve molluscs, the concentration of Cd has also been reported to be higher in the gills, and the elimination of this metal likely happens via the kidney (Guzmán-García *et al.* 2005, Raimundo and Vale 2008).

Lead (Pb)

Studies of the rivers and coastal lagoons of the Gulf of México have reported that the state of Veracruz presents one of the highest concentrations of Pb in the water with 212.18 mg L^{-1}, which exceeds the permissible limits for coastal waters (Guzmán-Amaya *et al.* 2005). However, the average concentration of Pb in oysters, *C. virginica*, from Tamiahua Lagoon in this study was 0.77 mg kg^{-1}, a value lower than in the lagoons at Llano (2.22 mg kg^{-1}) (Botello 1994), La Mancha (9.41 mg kg^{-1}) (Galaviz 2003), Mandinga and Alvarado (13.17 and 9.05 mg kg^{-1}, respectively) (Guzmán-Amaya *et al.* 2005), Tampamachoco (0.74 mg kg^{-1}) (Rosas *et al.* 1983), and Tamiahua (21.42 mg kg^{-1}) (Guzmán-Amaya *et al.* 2005). When compared to lagoons in the Gulf of México, the low concentration of Pb in *C. virginica* from the Tamiahua Lagoon in this study is most likely due to the absence of industrial activity, leading to the lower concentration of this metal in the water column and sediment (Apeti *et al.* 2005, Guzmán-Amaya *et al.* 2005). However, the earlier presence of these activities has promoted a 2000 % increase in this metal (Villanueva-Fragoso and Páez-Osuna 1996) in lagoons in Tabasco, Carmen and Términos in Campeche.

The Tamiahua Lagoon is classified as a RAMSAR site, although it has a high level of aquatic contamination due to untreated sewage, hydrocarbons, trash, solid waste, agrochemicals, and fertilizers. These inputs have resulted in elevated concentrations of copper, lead, cadmium, and chromium (Guzmán-Amaya 2005, Páez-Osuna 2005, RAMSAR 2006, 2008). However, the presence and concentration level of Pb in *C. virginica* depends on the bioaccumulation process which is a function of the bioavailability of the contaminant and 1) mobilization of the metals in interstitial water and their chemical speciation, 2) transformation of As, Hg, Pb, and Sn by processes such as biometilation, which affects solubility, volatility, and chemical properties, 3) distribution and abundance of sediment components (e.g. iron oxide and organic material), 4) competition between metals such as Cu, Ag, Zn, and Cd at points of entry into organisms, and 5) effects of bioturbation, salinity, the redox coefficient, and pH (Guzman-Amaya *et al.* 2005, Baqueiro-Cárdenas *et al.* 2007a).

As with Cd, there were higher concentrations of Pb in MMG than in GDG tissues for both males and females (0.49 mg kg^{-1} and 0.7 mg kg^{-1}, respectively) (**Table II**) (e.g. Oliver *et al.* 2001). This pattern of concentration (MMG>GDG) has been observed in other bivalve molluscs such as the blue mussel, *Mytilus edulis*, where the following pattern of concentrations by tissue has been reported: gills>digestive gland>foot>gonad (Mubiana and Blust 2007).

Arsenic (As)

The presence of As in Tamiahua Lagoon is primarily from forestry activity occurring in the adjacent region which combines 739 units of rural production, of which 49 are engaged in timber production (INEGI 2009). Arsenic is used as a fungicide, insecticide, herbicide, algaecide, rodenticide, and as a preservative applied directly on the wood (EPA 2003). An additional source of As in the Tamiahua region is runoff from petroleum exploration wells which are abandoned and not sealed (RAMSAR 2006, 2008).

The average concentration of As observed in *C. virginica* in this study was 7.36 mg kg^{-1}, which is less than 23 mg kg^{-1} reported for this species by Hernández *et al.* (2003) in the Bahía Cienfuegos of Cuba. Other studies on *C. virginica* in gulf coast waters of the United States have reported total concentrations of As to fluctuate between 4.1 and 39 mg kg^{-1} and which were attributed to the drainage of natural deposits of phosphates (Wilson *et al.* 1992). On the other hand, studies on the Japanese oyster, *C. gigas*, in coastal zones of southeast Taiwan and in Sonora, México, have shown As concentrations of 9.9 and 0.05 mg kg^{-1}, respectively (García and Ramos 2001, Liu *et al.* 2007, 2008). *C. gigas* and *C. virginica* share similar characteristics with physiological connections such as: they are sedentary organisms, they are strong bioaccumulators and have abundant populations, they have a relatively long average life expectancy, they are manageable and easily acclimatize to experimental conditions, and both have been used as environmental indicators (Rodríguez de la Rua *et al.* 2005). The concentration of As in *C. gigas* in coastal regions of the southwest of Taiwan was higher than in this study due to wastewater discharges containing high levels of inorganic As (Liu *et al.* 2008).

In the present study, significant differences in As concentrations were found between sampling sites, month, and sexes (**Table III**). These differences are likely due to the reproductive cycle which is influenced by changes in seasonal climate and the abundance of food that occurs during this cycle (Lango-Reynoso *et al.* 2000). In studies of Términos Lagoon, Campeche, Aguilar and Amador del Angel (2003) found that the condition index for *C. virginica* was negatively correlated with heavy metal concentrations in the tissues, and that the differences were associated with tissue function and exposure time to the heavy metals. These results support those obtained for *C. gigas* on the southwest coast of Taiwan where As concentrations followed a seasonal pattern of spring>summer>winter (Liu *et al.* 2008). Changes in temperature and salinity also induce the accumulation of As in *C. virginica* (Valette-Silver *et al.* 1999, Guzman-Amaya *et al.* 2005).

The differences between sampling sites are likely due to their location in the lagoon (**Fig. 1**). Sampling site 1, located in the navigation channel, between the island of Idol and the eastern part of Tamiahua municipality, receives inputs from human settlements located on the shores of the island. In contrast, sampling site 2 is in the center of the lagoon, between the islands of Idol and del Toro, is not directly impacted by any population. However, it is located near the mouths of the rivers San Jerónimo and Tancochi, which discharge livestock, agricultural, and urban waste from the adjacent municipalities. This site also is negatively impacted by abandoned oil wells due to its proximity (Catan I, II and Acamayas).

The same pattern of As, Cd, and Pb concentrations exist in tissues (i.e., MMG>GDG) of *C. virginica*, females had a higher average concentration of As (4.47 mg kg^{-1}) than males (3.64 mg kg^{-1}). Amiard *et al.* (1994) found a negative correlation between individual weight and the concentration of heavy metals. These observations coincide with the data obtained in this study; the highest concentrations of As were observed in spawned females, which had experienced a decrease in body mass.

CONCLUSIONS

MMG tissues from both males and females of *C. virginica* had the highest concentrations of Cd and Pb during the three seasons studied, although the highest concentrations of As were observed in females. These results can be explained in terms of the physiological processes of filtration and feeding with regard to sampling site location, sources of pollution, and weather conditions prevailing during each of the sampling periods (Leah *et al.* 2001).

The concentrations of Cd observed in this study exceeded permissible limits for human consumption established by public health standards for bivalve molluscs in México (SSA 1993), the European Economic Community (EEC) (FAO 1989), the United States of America (USA) (EPA 2003), and Spain. Concentrations of Pb did not exceed permissible limits. The existing regulations for health specifications in México regarding "fishery products, fresh and chilled bivalve molluscs", does not provide specifications on permissible concentrations of As. However, the NOM-129-SSA1-1995 (SSA 1995) suggests that for "fishery products: dry-salted. Provisions and specifications health", the level permitted for As in oysters is 2 to 4 mg kg^{-1} (**Table I**).

In México, the average consumption of oysters/person/day along coastal zones of the Gulf of México is approximately 12, with an approximate wet weight of 84 g (Lippmann 1992). The consumption of this quantity of oysters, considering the average concentration of Cd observed in this study, results in ingestion of 1.798 mg/day of Cd, a value that exceeds the permissible limits in the NOM-031-SSA1-1993 (SSA 1993) (**Table I**). Consequently, this metal is a strong biotoxicant and represents a public health risk in communities having a high oyster consumption (Fermín *et al.* 2003).

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