Rev. Int. Contam. Ambient. 21 (2) 55-62, 2005

MEAN LETHAL BODY CONCENTRATION OF CADMIUM IN *Crassostrea virginica* FROM A MEXICAN TROPICAL COASTAL LAGOON

Guadalupe BARRERA-ESCORCIA[1] e Irma WONG-CHANG[2]

[1]Departamento de Hidrobiología, UAM, Apartado Postal 55-535, Iztapalapa 09340, México, D. F. Estudiante del Programa de Doctorado en Ciencias Biológicas, UAM. Correo electrónico: gube@xanum.uam.mx
[2]Instituto de Ciencias del Mar y Limnología, UNAM, México, Apartado Postal 70305, Coyoacán 04510, México, D.F.

(Recibido octubre 2004, aceptado enero 2005)

Keywords: oyster, metals, bioconcentration, Mandinga lagoon

ABSTRACT

The acute effect of cadmium on *Crassostrea virginica* from the Mandinga Lagoon, México, was evaluated and related to corporal metal accumulation. The bioassay was performed with oysters exposed to lethal Cd concentrations, at temperature of 24.13 °C and salinity of 22.54 ‰. The exposed organisms accumulated from 261.20 to 508.80 μgCd/g dry weight in average. The metal incorporated in organisms exposed to the highest Cd concentration was superior in oysters which died during the experiment, than in survivors. However biococentration factor was lower in the highest Cd concentrations. There was weight loss associated to the exposition and it was greater in survivors. Deterioration could be related to a greater metabolic effort to compensate the entrance of Cd. The mean lethal concentration, $CdLC_{50\text{-}72h}$=24.87 mg/L, and the mean lethal body concentration, $CdLBC_{50\text{-}72h}$=502.25 μg/g (4.47 μmol/g), had good adjustments to the Probit model. The body concentration, the bioconcentration factor and the LBC_{50} associated to the physical and chemical parameter values, particularly salinity and temperature, could constitute useful tools in the evaluation of the cadmium adverse effects in Mexican coastal areas.

Palabras clave: ostión, metales, bioconcentración, laguna de Mandinga

RESUMEN

Se evaluó el efecto agudo del cadmio en *Crassostrea virginica* de la laguna de Mandinga y fue relacionado con la acumulación corporal del metal. El bioensayo fue llevado a cabo con ostiones expuestos a concentraciones letales de Cd a temperatura de 24.13 ºC y salinidad de 22.54 ‰. Los organismos expuestos acumularon de 261.20 a 508.80 μgCd/g de peso seco en promedio. La acumulación del metal en ostiones expuestos a la concentración más alta de Cd, fue superior en los ostiones muertos durante el experimento, que en los sobrevivientes. Sin embargo, el factor de bioconcentración fue menor en las mayores concentraciones de Cd. Hubo pérdida de peso asociada a la exposición y ésta fue mayor en los sobrevivientes. El deterioro podría relacionarse con un mayor esfuerzo metabólico para compensar la entrada de Cd. La concentración letal media $CLCd_{50\text{-}72h}$=24.87 mg/L y la concentración corporal letal media $CCLCd_{50\text{-}72h}$=502.25 μg/g

(4.47 µmol/g), tuvieron buen ajuste al modelo Probit. La concentración corporal, el factor de bioconcentración y la CCL_{50} asociadas a los valores de los parámetros fisicoquímicos, particularmente a la salinidad y a la temperatura pueden constituir herramientas útiles en la evaluación de los efectos adversos del cadmio en áreas costeras mexicanas.

INTRODUCTION

Metals are widely recognized as toxic agents and their effects have been evaluated in many species including mollusks (EPS 1990, Pawlisz *et al.* 1997). Cadmium adverse effects has been studied in mussels and oysters due to its toxicity and because mollusks are potentially useful as biomonitors (Ruelas-Inzunza and Páez-Osuna 2000). Recently some authors consider that metal concentrations in biota are better indicator of potential biological impact than concentrations in the environment because differences in metal bioavailability are automatically taken into account (Borgmann 2000). The bioavailable fraction constitutes the greatest danger to biota (Soto-Jiménez *et al.* 2001). Cadmium concentrations are increased in several coastal lagoons of the Gulf of México (Villanueva and Botello 1998). The presence of Cd in marine sediment fluctuates from < 1 mg/kg, in relatively pristine environments, to > 31 mg/kg in contaminated areas (Sadiq 1992); high values detected in polluted rivers are associated to organic matter and industrial complexes, and Cd is easily distributed in aquatic ecosystems because its great solubility (Villanueva and Páez 1996). The Mandinga lagoon (19°00'-19°06' N and 96°02'-96°06' W) at the Veracruz State (Contreras and Castañeda 1995), is a typical tropical coastal lagoon with a relative impact of human activities. There is not a punctual source of Cd in Mandinga lagoon, but water concentration is over the limits, sediment content was 0.015 µgCd/g dry weight (Rosas *et al.* 1983) and 1.22 µgCd/g dry weight (Botello 1994), and oysters contents varied from 1.54 µgCd/g dry weight (Rosas *et al.* 1983) to 3.13 mgCd/g dry weight (Villanueva y Botello 1998). In that manner, Cd represents a potential risk to biota in the lagoon. This is shelter of species as the oyster *Crassostrea virginica* that represents an important fishery resource for many communities (Palacios-Fest and Vargas-Rangel 2002). There is knowledge about the oyster sensibility acclimated to temperatures as 16 ºC or 24 ºC (Shumway 1996), exposed to different substances including cadmium (Roesijadi 1996). However, *C. virginica* in a tropical environment as Mandinga lagoon, lives among 28 ºC and 33 ºC, and acclimation temperature is associated to the physiological responses. In México, studies on the accumulation and the aquaculture aspects for its commercial exploitation have been made (Secretaría de Pesca 1988). The management of the species usually involves the fertilization in farms and the posterior introduction of juveniles in the lagoons. However, little is known about its responses to environmental toxic agents.

Therefore the aim of this study was to assess the lethal effect of cadmium on the oyster *Crassostrea virginica* from a Mexican tropical coastal lagoon and its relationship with the body concentration.

MATERIALS AND METHODS

Oyster collection and maintenance in laboratory

Commercial sized oysters (>5 cm length) samples were collected in the months of September 1997, November 1997, and September 1998 in the Mandinga Lagoon. Measurements *in situ* included: dissolved oxygen (0.01 ± 0.005 mg/L), temperature (0.1 ± 0.05 ºC), pH (0.01 ± 0.005 unities) and salinity (0.1 ± 0.05 ‰) with a Horiba U-10 multianalyzer. Oysters were transported in plastic bags at 4 °C to the laboratory where the epibiontes were removed according to Eaton *et al.* (1995). Oysters were maintained in a closed recirculated aquaria system (600 L) provided with charcoal-anthracite and biological filters, with Instant Ocean artificial saline water (Shumway and Koehn 1982). Temperature was maintained at 25 ± 1 °C and aeration kept constant (>5 mg/L of dissolved oxygen), salinity was adjusted at a ratio of 0.5 ‰ per day until the assay salinity (22 ‰) was reached. *Tetraselmis suecica,* cultivated in Gillard f-2 medium was supplied as food, 15 to 20 X 10^6 cells per day per organism (Castrejón *et al.* 1994) during the five weeks of the maintenance period.

Toxicity tests

Semistatic 168 h bioassays were conducted in ten aquaria of glass, with a volume of 40 L. Aeration kept constant, temperature was controlled with thermostat to 25 ± 1 ºC. Water was prepared to 22 ‰,

24 h before, to insure the same salinity and pH in the aquaria. During the assay physical-chemical parameters were recorded daily. Oysters were selected morphometrically homogeneous with 68.9 mm length ± 9.0 standard deviation (SD), 41.3 g total weight ± 7.92 SD, and with a good Condition Index (CI) of 101.7 g/mL, calculated as (dry weight X 1000/ shell) cavity volume (Walne 1984), using eight organisms per concentration, in duplicate. Feeding was suspended 24 h before the assay started. Oysters were exposed to: 5.75, 13.05, 18.85 and 30.50 mg/L. Cadmium was supplied from a stock solution of 50 g/L of $CdCl_2$•2 ½ H_2O (Baker Analyzed) in the desired volume to reach the experimental concentrations. A control group without cadmium was considered. Partial water exchanges, corresponding to 25 % of the water volume, were made every 48 h and cadmium concentration was adjusted to maintain the same level. Total ammonium was measured according to Lind (1985) with the indophenol's blue technique (sensibility of the method 2 µg/L) and maintained below 0.1 mg/L. Mortality was recorded every 2 h during the first day and later on every 24 h. Death criterion was set at permanent opening of the valves. Morphometry was taken after the assay (length, total weight, wet and dry weight). The CI was calculated in the survivors. The dry weight expected in oysters of the same size, was calculated with the morphometric equation obtained by Powell *et al.* (1992): length $(mm)_j$ = dry weight $(g)_j^{0.317}$•$10^{0.669}$ and it was compare with the obtained dry weight.

Mean lethal concentration (LC_{50}) and mean lethal body concentration (LBC_{50}) were established through the Probit method using the DORES Program (Ramírez 1989). The Probit transformation (log normal) is one of the most frequently assumed to estimate the mortality response. It is the fitting model of the distribution of mortality against the log of exposure concentration (and the tissue accumulation in this case), to generate a straight line (Newman and Dixon 1996). The line obtained is significant if confidence limit is lower than LC_{50} and positive, coefficient of determination is significant with $p<0.05$, coefficient of correlation is over 0.8, coefficient of variation is under 15 %, and if χ^2 observed is lower than χ^2 expected (Ramírez 1989). The Bioconcentration Factor (BCF) was calculated as final tissue concentration/water concentration (Buikema *et al.* 1982).

Cadmium measurements

Total cadmium was evaluated in water samples extracted from each aquaria. They were obtained six hours after the addition of Cd when experiment began, at 96 h, as well as in the end (168 h). The water was digested with nitric acid in a microwave oven (CEM-MDS-81D); at the end of the assay the oysters tissue was digested according to the method proposed by Huan (1994). Cd concentrations were determined by atomic absorption spectrophotometer provided with acetylene-air flame Varian AA20 (0.01 ± 0.005 mg/L). Water samples of the Centro Nacional de Metrología with 1.45 ± 0.06 mg Cd/L were used as reference.

Statistical analysis

The analysis was performed with the Statistica software, Statsoft Ser. 1997, to compare the initial and final values (t test), the differences among control and exposed organisms (ANOVA) and to associate the metal concentrations and the morphometric characteristics (coefficient of correlation). The standard deviation (SD) was obtained. The significance of the tests was $p<0.05$.

RESULTS

Collected oysters had an average of 2.30 µgCd/g dry weight ± 1.11 SD. During samplings *in situ* parameters were: temperature 25.7 ± 3.6 °C, salinity 14.15 ± 4.85 ‰, pH 7.66, and dissolved oxygen 5.91 ± 1.30 mg/L.

During the bioassay the concentrations in the control aquaria were 0.01 mgCd/L in average, from below the detection level to 0.01 mgCd/L. The physicochemical parameters were: temperature 24.1 ºC ± 0.6 SD, salinity 22.5 ‰ ± 0.6 SD, pH 8.33 ± 0.30 SD, and dissolved oxygen 3.93 mg/L ± 1.17 SD. The ammonium concentration was 0.019 mg/L ± 0.009 SD at the beginning of the assay, it increased to 0.087 mg/L ± 0.023 SD at the end of experiment. The physicochemical properties in aquaria were controlled during the maintenance, and they were similar in duplicates during the experimental period. In that manner, data of duplicates were considered together (16 organisms per concentration) to make the analysis.

The CI in control oysters diminished from 101.7 g/mL to 48.1 g/mL as result of the starvation. The CI of exposed organisms was not determinated because the survivors did not close the valvae completely and their intervalvae liquid was lost. In all the organisms total, wet and dry weight diminished, but the exposed oysters weight was lower than controls (**Fig. 1**). The expected dry weight calculated for oysters of the

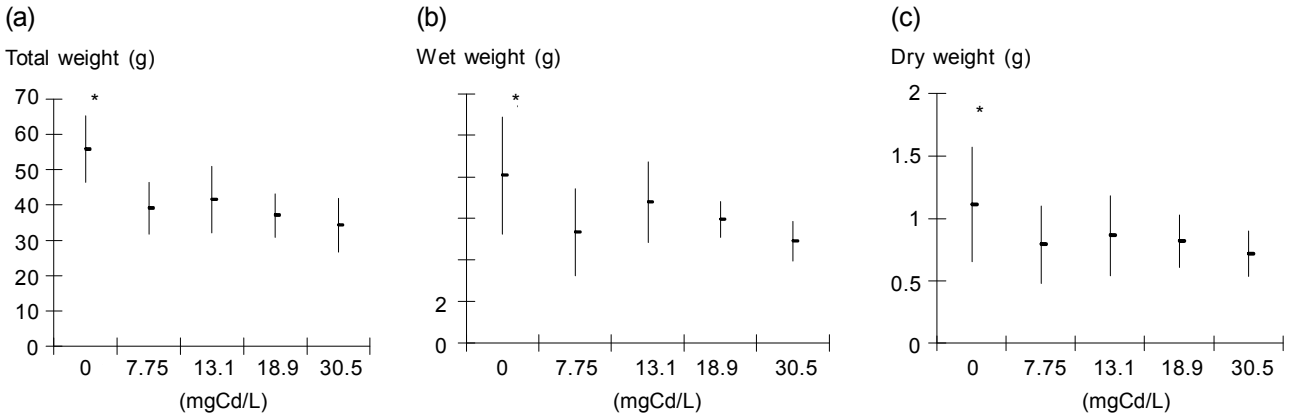

Fig. 1. Oysters morphometry after the bioassay. Significant differences (*) among control and exposed oysters indicated that weight loss was a result of the Cd exposition

same size was 1.71g. After the assay dry weight was 1.11 g ± 0.46 SD in the control oysters and it was 0.84 g ± 0.25 SD in the exposed organisms. The loss represents, in average 30 % in controls, and 50 % in exposed organisms. The increase of cadmium concentration in tissue presented significant inverse relationship with the total, wet and dry weight (r = -0.48, r = -0.34 and r = -0.35, respectively). In addition, there was an important loss weight in survivors, proportional to the water cadmium concentration (r= -0.96).

The oysters exposed to 5.75 mgCd/L that died before 72h, incorporated 261.20 μgCd/g dry weight and had a BCF = 45.39, whereas the organisms exposed to 30.50 mg/L showed values of 508.80 μgCd/g in tissue (**Fig. 2a**) and had a BCF = 16.68 (**Fig. 3**). The concentration in oysters increased with the water concentrations (r = 0.90), meanwhile the BCF diminished (r = -0.96). At the end of the assay (168 h), the survivor oysters exposed to 5.75 mgCd/L incorporated 158.00 μgCd/g (BCF = 27.48), and those exposed to 30.50 mgCd/L incorporated 113.20 μgCd/g (BCF = 3.71). The accumulation in survivors (168 h) was lower, there was not clear relationship among the incorporation in tissue and concentration in water (**Fig. 2b**), and the BCF was 3.76. However, the general balance (168 h) considering all the specimens, indicated that cadmium accumulated in the oysters presented a negative relationship with the BCF (r= -0.62) (**Fig. 3**).

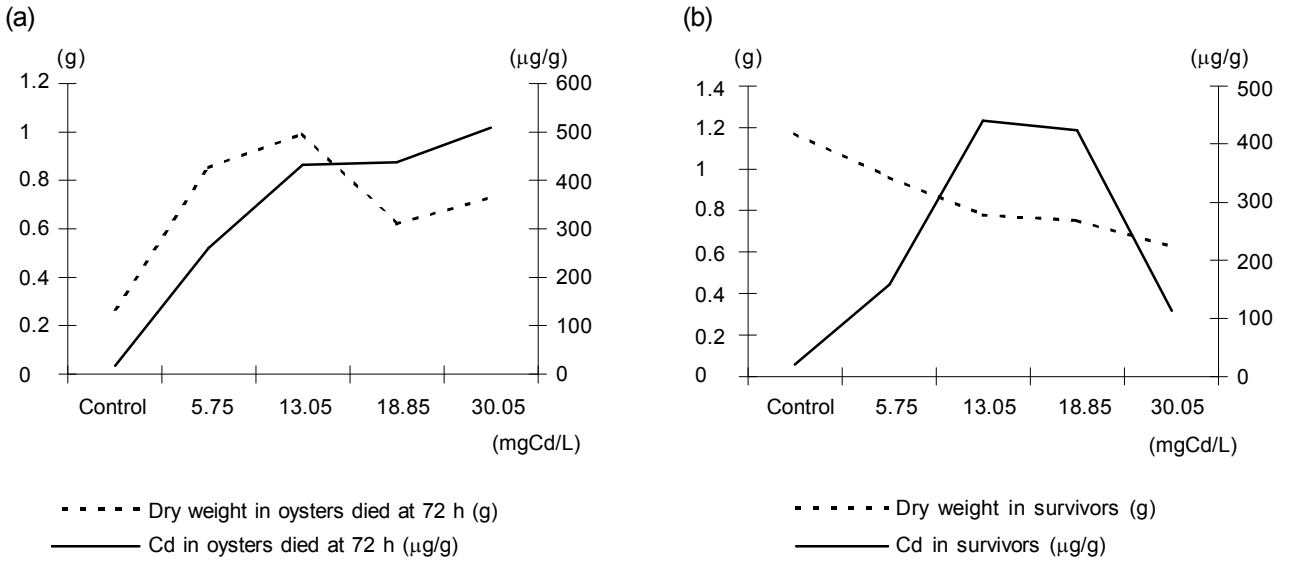

Fig. 2. Cd in tissue of oysters died at 72 h proportional to the water concentration without important weight loss, and Cd concentrations in survivors not related to water concentrations but with important weight loss

TABLE I. ADJUSTMENT FROM THE PROBIT LOG X MODEL WITH LETHAL CONCENTRATION AND LETHAL BODY CONCENTRATION

	Water concentration	Body concentration
Cd lethal concentration$_{50,72h}$	24. 87 mg/L	502.25 μg/g (4.47 μmol/g)
Coefficient of determination r^2	0.94	0.65
Coefficient of correlation	0.97	0.81
Coefficient of variation (%)	12.06	7.38
$\chi^2_{(0.05,3)}$ Obtained and expected	0.95< 5.99	4.73 < 5.99

The Cd LC50-72h was 24.87 mg/L and the Cd $LBC_{50\text{-}72h}$ was 502.25μg/g (4.47 μmol/g). The Probit model was significant in both cases ($p<0.05$). The variation coefficient (VC) was under 15 %, but the lower VC was obtained using the corporal concentrations. For this reason the adjustment could be considered better with the lethality relationship based in the oyster concentrations. On the other hand, the correlation coefficient with mortality was higher using the water concentration (r = 0.97) than with the body concentration (r = 0.81) (**Table I**).

Values of LC_{50} calculated at 48, 72, 96 and 120 h, did not show a linear behavior, meanwhile the LBC_{50} obtained for the same periods had almost a linear behavior (**Fig. 4**).

DISCUSSION

Several species of the genus *Crassostrea* are considered bioaccumulator of metals: *C. iridisens* is moderate (Soto-Jiménez *et al.* 2001), and *C. corteziensis* and *C. rhizophorae* are net accumulators of Cd (Ruelas-Inzunza and Páez-Osuna 2000, Silva *et al.* 2001). The accumulation in died oysters at 72 h in the present study (261.20 to 508.80 μg/g), was proportional to the water concentration, the organisms could not regulate the entrance of cadmium and they accumulated the metal. The accumulation generated values ten times greater than values measured in oysters *C. virginica* (45.4 μg/g) exposed to Cd in the Patuxent River (Abbe *et al.* 2000). The Cd in *C. virginica* has been related to external concentration and time of exposure. Zaroogian (1980) registered 125 μgCd/g in *C. virginica* exposed to 0.005 mgCd/L, and 130 μgCd/g exposed to 0.015 mgCd/L, after 36 weeks. Meanwhile Zaroogian and Morrison (1981) registered 91 μgCd/g in *C. virginica* exposed to 0.005 mgCd/L during 37 weeks. The Cd incorporated during the assay in short time, but in higher water concentrations could be considered similar to the accumulation in other bivalves, as *Andara granosa* that accumulated 350 μgCd/g exposed to water from Vashi, India, with 2.4 mgCd/L after 96 h, *A. rhombea* reach 305 μgCd/g and *Perna viridis* 240 μg/g in water with 1.8 mgCd/L (Patel and Anthony 1991) and the accumulation in *Mytilus edulis* was 300-500 μgCd/g, exposed to 0.2 mgCd/L (Köhler

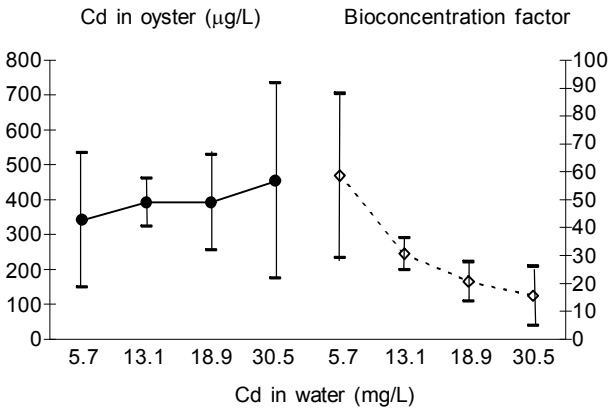

Fig. 3. Increase in Cd concentrations in oysters and Bioconcentration Factor (BCF)

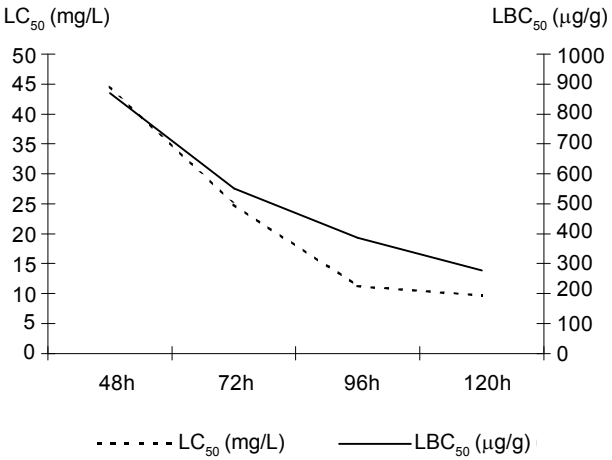

Fig. 4. Mean Lethal Concentration (LC_{50}) and Mean Lethal Body Concentration (LBC_{50}) obtained in different hours for *Crassostrea virginica* exposed to Cd

and Riisgard 1982). BCF diminished in higher Cd concentration. Organisms did not incorporate the metal as fast as other exposed to lower concentrations. Cd interferes with osmoregulation, oxygen consumption, modifies gill structure, and inhibits Ca influx (Sadiq 1992). In that manner respiratory and ingestion activities most to be done associated to a greater energetic cost when organisms are exposed to Cd. This can be the reason of the higher weight loss in exposed oysters. The lower Cd accumulation in survivors indicates a capacity of regulation with a metabolic expense, represented by the dry weight loss.

The CI indicates that organisms began the bioassay in adequate condition; their feeding was sufficient during the maintenance period, IC diminution was consequence of starvation, the weight loss indicated a deterioration in general condition of the organisms. But differences among control and exposed organisms indicate that cadmium incorporation was associated to a greater weight loss. An inverse relationship between cadmium concentration in tissue and condition index has been recorded in natural oyster populations in the Patuxent River (Riedel *et al.* 1998). Traditionally effects are related to external concentration. Actually, several authors coincide in the convenience to associate the body concentration with the effect. Walker and Gobas (1999) indicate that protocols of environmental protection should include the measurement of the internal concentration as well as the exposure concentration associated to the toxic effect. Borgmann (2000) indicates that the body burden approach is better than the analyses with the water exposure concentration, particularly with non essential metals, as cadmium.

The interpretation based in water concentration allows a comparison with the traditional studies, as the Calabrese *et al.* (1973) determination of the Cd LC_{50}-48h for embryos of *C. virginica* and other studies with adults (Jorgensen *et al.* 1991), but the Cd accumulation and the damage associated to selected experimental parameters, gave information about the native oyster population sensitivity. In the present study, the LBC_{50} and the LC_{50} results were significant to the model and the fit was similar. Both of them are useful to make an interpretation of the damage, and to give complementary information. However the LBC_{50} calculated in several periods showed a linear behavior comparing with the LC_{50} as shows the **figure 4**.

Studies on the lethal effect of metals on *C. virginica* are not so frequent in the present as in the past, concentrations of 1.0 mg Cd/L in seawater are considered extremely high to note effects by exposition in *C. virginica* (Roesijadi 1996), but environment conditions can be modify and the solubility of the metal in water can increase. The Cd in sediment has a relative motility to the water column. The movement is influenced by several factors, the polluted sediments can remain as a source of metals after their input to the environment has ceased (Ford *et al.* 1998). Previous studies had measured 0.015 µg Cd/g in sediment of the Mandinga lagoon (Rosas *et al.* 1983) and recent studies had determinate 1.09 µgCd/g with 0.80 µgCd/g bioavailable (Villanueva and Botello 1998). Complexation of Cd with Cl is common in seawater, but the soluble form Cd^{2+} increases with the decrement in salinity (Sadiq 1992). The salinity condition in tropical coastal lagoons varies great deal along the year. Senthilnathan and Balasubramanian (1998), consider that Cd concentrations in *C. madrasensis* are correlated with environmental changes as the monsoon season and Cheggour *et al.* (1999), indicates that Cd in *C. gigas* is related to several ecological factors. The behavior of cadmium associated with the water salinity and temperature might play an important role in the bioavailability, and the oyster responses are influenced by environmental changes of both parameters too; *C. virginica* in water of Patuxent River with 0.08 ± 0.03 mgCd/L, 35.3 ºC and 16 ‰ incorporated 45.4 ± 4.3 µg Cd/g (Abbe *et al.* 2000). The bioassay selected salinity (22.5 ‰) is the average value in the Mandinga lagoon and the temperature (24.1 ºC) is considered adequate to the oyster culture (Secretaría de Pesca 1988). However, the Mandinga lagoon salinity fluctuates between 4.6 to 29.0 ‰ and the temperatures are between 28.2 to 33.1 ºC, the dissolved oxygen is found between 1.73 and 4.22 mg/L, and the pH from 4.5 to 8.7 (Contreras and Castañeda 1995). The natural variation in physical-chemical parameters on the lagoon may play an important role in the Cd accumulation in oyster. Considering the natural variation, samplings coincide with the rainy season, when oysters begin the increase of reserves for winter. During the cold season oysters accumulate higher glycogen reserves, and they have a better condition, but spawning can generate irregularities in the organisms. Spawning is not a unique event, it can occur from cool to warm season, several times, with the increase of temperature. During spring and summer oysters condition varies, and organisms gonad can be present or not. We consider that, from September to November, there is a certain homogeneity in the oysters condition.

Previous studies indicated that total cadmium con-

centration in water of the Mandinga lagoon was 0.002 mgCd/L and in oyster 1.54 µgCd/g (Rosas *et al.* 1983). A recent study detected 3.13 µgCd/g (Villanueva and Botello 1998). In the present study the cadmium concentration in oysters collected in the Mandinga Lagoon was 2.3 µgCd/g ± 1.11 SD. It is consider similar, because the dispersion of data, and one extreme case of 4.2 µgCd/g. The resistance of *C. virginica* native from Mandinga lagoon was similar to organisms from temperate latitudes, but the cadmium effects in lower concentrations should be known due tropical coastal lagoons have higher temperatures and particular behavior. The incorporation is related to the environmental conditions, the concentration in water and sediment, and the time of exposition. On the other hand, the LBC_{50} could represents a useful tool in the evaluation of cadmium effects in native populations.

Research must be continue to evaluate adverse effects of metals to *C. virginica* considering normal environmental variations in order to assess the risk for this as well as for other commercial species in the coastal areas of Mexico.

ACKNOWLEDGMENTS

This research was supported by the National Council of Science and Technology (CONACyT) under the project "Toxicity of chromium and cadmium in the oyster *Crassostrea virginica* from the Mandinga Lagoon, Veracruz" (0636P-T). We thank Ricardo Rosas Cedillo for the analyses of metal samples and Alfonso V. Botello, Concepción Gutiérrez, Cecilia Vanegas, Patricia Ramírez and Juan Manuel Hernández for the critical review of the manuscript.

REFERENCES

Abbe G. R., Riedel G. F. and Sanders J.G. (2000). Factors that influence the accumulation of cooper and cadmium by transplanted eastern oysters (*Crassostrea virginica)* in the Patuxtent River, Maryland. Mar. Environ. Res. 49, 377-396.

Borgmann U. (2000). Methods for assessing the toxicological significance of metals in aquatic ecosystems: bioaccumulation-toxicity relationships, water concentrations and sediment spiking approaches. Aquatic Ecosystem Health and Management 3, 277-289.

Buikema Jr. A.L., Niederlehner B.R. and Cairns Jr. J. (1982). Biological Monitoring. Part IV Toxicity Testing. Water Res. 16, 239-262.

Calabrese A., Collier R.S., Nelson D.A. and MacInnes J.R. (1973). The toxicity of Heavy Metals to Embryos of the American Oyster *Crassostrea virginica*. Mar. Biol. 18, 162-166.

Castrejón O.L., Porras D.D. and Band S.Ch. (1994). *Cultivo de alimento vivo para acuicultura*. Instituto Nacional Indigenista-Universidad del Mar, Oaxaca, 118 p.

Cheggour M., Chafick A., Texier H., Bouhallaoui A., Gharbi N., Boumezzough A. and Elattar J. (1999). Bioaccumulation of some metallic elements by the cultivated oyster *Crassostrea gigas* (Thunberg) in Oualidia Lagoon (Morocco); the role of ecological factors. Haliotis 28, 31-44.

Contreras E.F. and Castañeda C.O. (1995). *Los ecosistemas costeros del Estado de Veracruz*. Gobierno del Estado de Veracruz. Secretaría de Desarrollo Agropecuario Forestal y Pesquero, México, 81-88.

Eaton A.D., Clesceri L.S. and Greenberg A.E. (Eds.) (1995). *Standard methods for the examination of water and wastewater*. American Public Health Association, Maryland, 8-1 – 8-47.

Environmental Protection Series (EPS). (1990). *Guidance Document on Control of Toxicity Test Precision Using Reference Toxicants. Report EPS1/RM/12*. Canada, 85 p.

Ford T., Sorci J., Ika R. and Shine J. (1998). Interactions between metals and microbial communities in New Bedford, Massachusetts. Environ. Health Perspect. 106 (Supl. 4), 1033-1039.

Huan S.Ch. (1994). *General Guidelines for the Acid Digestion of Fish and Shellfish. Reference 83 Method Number NIEA C303 01T.* Executive Yuan Environmental Protection Agency. Republic of China. Official Gazette 7, 1-6.

Jorgensen S.E., Nielsen S.N. and Jorgensen L.A. (1991). *Handbook of ecological parameters and ecotoxicology.* Elsevier, New York, 523-524.

Köhler K. and Riisgard H.U. (1982). Formation of metallothioneins in relation to accumulation of cadmium in the common mussel *Mytilus edulis*. Mar. Biol. 66, 53-58.

Lind O. (1985). *Handbook of common methods in limnology.* Kendal/Hunt, 199 p.

Newman M.C. and Dixon Ph.M. (1996). Ecologically meaningful estimates of lethal effect in individuals. En: *Ecotoxicology. A hierarchical treatment.* (M.C. Newman and Ch.H. Jagoe, Eds.). Lewis Pubs., New York, pp. 225-253.

Patel B. and Anthony K. (1991). Uptake of cadmium in tropical marine lamelibranch and effects on physiological behavior. Mar. Biol. 108: 457-470.

Palacios-Fest M. and Vargas-Rangel R. (2002). Pesquería

de ostión. En: *La pesca en Véracruz* (A.P. Guzmán, B.C. Quiroga, L.C. Díaz, C.D. Fuentes, C.M. Contreras and L.G. Silva, Eds.). Secretaría de Agricultura, Ganadería y Desarrollo Rural, Pesca y Alimentación, Instituto Nacional de la Pesca y Universidad de Veracruz, México, pp. 235-241.

Pawlisz A.V., Kent R.A., Schreider U.A. and Jefferson C. (1997). Canadian water quality guidelines for chromium. En: *Environmental toxicology and water quality. An International Journal CCC 1053-4725/97/020/23-6.1* (D.L. Liu and B.J. Dutka, Eds.). J. Wiley & Sons. Ontario, pp. 123-183.

Powell E.N., Hofmann E.E., Kinck J.M. and Ray S.M. (1992). Modeling oyster populations I. A commentary on filtration rate. Is faster always better?. J. Shellfish. Res. 11, 387-398.

Ramírez A. (1989). Programa para el Curso Regional de entrenamiento sobre ensayos biológicos y pruebas de toxicidad como bases técnicas para formular un Criterio de Calidad de agua costera en la región del Gran Caribe. Proyecto Regional. Colombia. INDERENA/PAC/PNUMA/FAO/COI., 24 p.

Riedel G.F., Saudersd J.G. and Breitburg D.L. (1998). The effects of multiple stressors on estuarine ecosystems: the influences of nutrient enrichment on trace element cycling. 19th Meeting Society of Environmental Toxicology and Chemistry Abstracts, N.C., pp. 26.

Roesijadi G. (1996). Environmental factors: response to metals. En: *The eastern oyster Crassostrea virginica* (V.S. Kennedy, R.I.E. Newell and A.F. Eble, Eds.). A Maryland Sea Grant Book College Park, Maryland, pp. 515-537.

Rosas I., Báez A. and Belmont R. (1983). Oyster (*Crassostrea virginica*) as an indicator of heavy metal pollution in some lagoons of the Gulf of Mexico. Water Air Soil Pollut. 20, 127-135.

Ruelas-Inzunza J.R., and Páez-Osuna F. (2000). Comparative bioavailability of trace metals using three filter feeder organisms in a subtropical coastal environment. (Southeast Gulf of California). Environ. Pollut. 107, 437-444.

Sadiq M. (1992). *Toxic metal chemistry in marine environments*. Marcel Dekker, N.Y., 390 p.

Secretaría de Pesca (1988). *Manual Técnico para la Operación de Centros Acuícolas de Producción de Ostión*. Secretaría de Pesca, México, 324 p.

Senthilnathan S. and Balasubramanian T. (1998). Heavy metal concentration in oyster *Crassostrea madrasensis* (Bivalvia/Anisomyaria) from the Uppanar, Vellar and Kaduviar estuaries of Southeast coast of India. Indian J. Mar. Sci. 27, 211-216.

Shumway S.E. (1996). Natural environmental factors. En: *The eastern oyster Crassostrea virginica* (V.S. Kennedy, R.I.E. Newell and A.F. Eble, Eds.). A Maryland Sea Grant Book College Park, Maryland, pp. 467-503.

Shumway S.E. and Koehn R.K. (1982). Oxygen consumption in the american oyster *Crassostrea virginica*. Mar. Ecol. Prog. Ser. 9, 59-68.

Silva C.A.R., Rainbow P.S., Smith B.D. and Santos Z.L. (2001). Biomonitoring of trace metal contamination in the Potengi Estuary, Natal (Brazil), using the oyster *Crassostrea rhizophorae*, a local food source. Water Res. 35, 4072-4078.

Soto-Jiménez M., Paez-Osuna F. and Morales-Hernández F. (2001). Selected trace metals in oysters (*Crassostrea iridiscens*) and sediments from the discharge zone of the estuarine sewage outfall in Mazatlan Bay (southeast Gulf of California): chemical fractions and bioaccumulation factors. Environ. Pollut. 114, 357-370.

Villanueva F.S. and Botello A.V. (1998). Metal pollution in coastal areas of Mexico. Rev. Environ. Contam. Toxicol. 157, 53-94.

Walne P.R. (1984). *Cultivo de moluscos bivalvos*. Acribia, Zaragoza, 206 p.

Walker S.L. and Gobas F.A.P.C. (1999). An investigation of the application of the Canadian water quality guidelines. Environ. Toxicol. Chem. 18, 1323-1328.

Zaroogian G.E. (1980). *Crassostrea virginica* as an indicator of cadmium pollution. Mar. Biol. 58, 275-284.

Zaroogian G.E. and Morrison G. (1981). Effect of cadmium body burdens in adult *Crassostrea virginica* on fecundity and viability of larvae. Bull. Environ. Contam. Toxicol. 27, 344-348.