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Lindane and Endosulfan Sulfate Isomers in Crassostrea virginica (Gmelin, 1791) Oyster Populations in Lagoon Systems from Central Gulf of Mexico

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***Abstract*—** *The aim of this study was to determine Lindane and Endosulfan Sulfate isomers in Crassostrea virginica oyster populations (Gmelin, 1791) in the Mandinga and Alvarado lagoon systems located in the central Gulf of Mexico. Samples were taken from the main oyster banks of each lagoon system, during the three representative seasons of the region, wet, dry and north winds. By means of free diving, 30 commercial size oysters (7 ± 3 cm) were collected in four oyster banks or stations of the Mandinga lagoon system, totaling 360 organisms, while in the Alvarado lagoon system there were a total of 90 oysters during the annual cycle. Concentration of lindane and endosulfan sulfate isotopes in C. virginica was performed with a gas chromatograph (Thermo Electron Model Trace GC Ultra 115V, Thermo Fisher Scientific Inc©, Monterrey, Nuevo León, México) with an Electron capture detector. Results showed that in the Alvarado Lagoon system mean concentrations of C. virginica oysters for lindane pesticide were 4.11 ± 3.83 ng·g^{-1}, whereas for the Mandinga lagoon system, were 8.69 ± 5.15 ng·g^{-1}. Endosulfan sulfate showed the highest average concentration in the Mandinga lagoon system with 24.68 ± 1.20 ng ·g^{-1}. In addition, the endosulfan sulfate presents differences in its spatial distribution; high concentration levels in the Mandinga lagoon system whereas the lindane heterogeneity at all sampling points in both lagoons. Values of concentrations and relationships between compounds suggest recent contributions that could correspond to the excessive fluctuations of water discharged into the lagoon caused by the atypical rains of the year of sampling. It was concluded that endosulfan sulfate and lindane show concentration in all the points of sampling in both lagoons.*

***Keywords*— coastal lagoons, Crassostrea virginica, endosulfan sulfate, lindane, toxicity.**

I. INTRODUCTION

Oyster *Crassostrea virginica* (Gmelin, 1791) is distributed from the Gulf of St. Lawrence, Canada to the coasts of Brazil and Argentina [1-3]. In Mexico, this fishery is mainly exploited in the coastal lagoons of the Gulf of Mexico. In 2014, the state of Veracruz recorded the highest oyster production, a result of the cooperative work of Tamiahua, Pueblo Viejo, Mandinga and Alvarado lagoons [4]. However, organisms that inhabit the Mandinga and Alvarado lagoon systems, located in the central area of the Gulf of Mexico, are subject to constant anthropogenic nature stress, which in turn has caused pollution issues in these important systems.

The Mandinga lagoon system is influenced by the Jamapa river [5], while the Alvarado lagoon system receives it from the Papaloapan River and Blanco River. These three rivers discharge in their slopes what they in turn receive as an impact of industrial and agricultural activities, which end up deposited in the aforementioned lagoon systems. Agricultural crops are part of the process of pesticide mobility, due to their retention properties, which modify the process of exchange of volatile substances between soil and air, which ultimately reach the lagoon systems. In agriculture, products with active substances known as organochlorine pesticides are applied [6,7], among the most used today is lindane, which is characterized by being long-lived and persistent. Organic matter is an important factor in the adsorption of lindane;

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an increase in organic matter increases persistence, while increasing solubility results in increased mobility of lindane. From the degradation of hexachlorocyclohexane (HCH) results in (α-HCH, β-HCH and γ-HCH) from these isomers γ-HCH is known as lindane [8].

Another important pesticide is endosulfan, a toxic compound that has been widely used in Mexico. Endosulfan has been found to produce neurotoxic, hematotoxic and nephrotoxic effects in mammals and is highly toxic to aquatic organisms. Endosulfan is an organochlorine pesticide consisting of a mixture of the isomers endosulfan (alpha) and endosulfan (beta). The National Institute of Ecology (INE) in Mexico, highlights in its "Diagnosis on the endosulfan status in Mexico" conducted in 2011, that endosulfan belongs to the group of cyclodienes and is chemically similar to aldrin, chlordane and heptachlor. Its main metabolite is endosulfan sulfate, it can be found in the environment by photolysis of endosulfan or as a result of its oxidation by microorganisms. Lindane and endosulfan sulfate are persistent, toxic, and bioaccumulative isomers that can travel great distances. As a consequence, they are proposed to be included in the Stockholm Convention [9,10]. The *C. virginica* oyster is a benthic organism which due to its development, feeding and reproduction system is in contact with different compounds that bioaccumulate and biomagnify in trophic chains until reaching human consumption [11-14], and is used as a biomarker [15]. The objective of this study was to determine the concentration of Lindane Isomers and Endosulfan Sulfate in oyster populations *Crassostrea virginica* (Gmelin,1791) in lagoon systems from central Gulf of Mexico to determine if there are potential risks to public health due to their consumption.

II. MATERIALS AND METHODS

This study was developed in the Mandinga lagoon system located between 19° 00'-19°06'N and 96°02'-96°06'W while the Alvarado lagoon system is between 18°44'-18°52'N, 95°44'-95°57'W. Both systems are located in the central zone of the Gulf of Mexico, (Fig. 1). It is shown, in both lagoon systems, the main oyster banks where the sampling work was carried out. This banks were chosen for their production capacity.

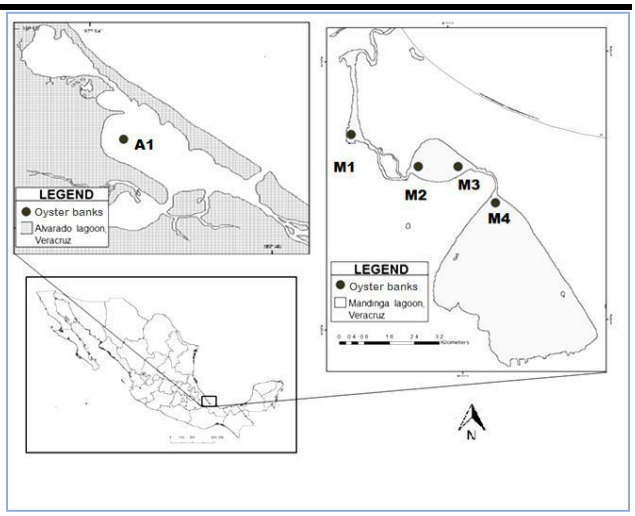

Fig.1: Oyster banks located in the Mandinga and Alvarado lagoon systems in the central area of the Gulf of Mexico.

Those works were carried out in three representative seasons of the region, wet, dry and north winds. By means of free diving, 30 commercial size oysters (7 ± 3 cm) were collected in the Mandinga lagoon system in four oyster banks or stations, totaling 360 organisms, while in the Alvarado lagoon system there were a total of 90 oysters. Organisms were removed and cleaned at each sampling station. They were transported in accordance with NOM-109-SSA1-1994 (2003) [16], to the Research and Aquatic Resources Laboratory (LIRA) of the Technological Institute of Boca del Río (ITBOCA) to carry out the cleaning and conservation process of the oyster samples. Oysters were cleaned with running water to remove excess sludge and adhered particles. Subsequently, the oyster muscle tissue was extracted with the use of a de-sheller, as well as the removal of the intervalvar liquid. Once this soft tissue was removed, it was deposited in previously labeled Ziploc® sealed bags. Immediately, these were placed in a deep freezer in order to continue the drying process. Frozen samples were dehydrated in a Thermo Savant Moduly OD-114 lyophilizer for 72 hours at -49 °C and a pressure of $36x10^{-3}$ mbar. After lyophilization, samples were ground in an Osterizer blender until a fine particle size. Subsequently, these samples were stored inside a desiccator with silica gel, to avoid possible absorption of moisture as well as samples fungal contamination. The analytical material used in the study was prepared following the analytical protocol for pesticide residues described by Waliszewski *et al*. (2008) [17]. The glassware used for this process was washed with 10% phosphate free neutral soap, then by potassium dichromate and rinsed with potable water. Thereafter, it was rinsed with distilled water Milli-Q grade, petroleum ether and acetone to eliminate all residues of phosphates and fats. Subsequently it was drained and dried with

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airflow. Once dried, they were stored in Ziploc® sealed bags and then in properly labeled containers with lid. 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. In order to analyze the pesticides lindane and endosulfan sulfate in oyster samples, it was used hexane (Backer) with a boiling temperature range of 40-50 °C and sodium sulfate powder (Backer) 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) with a purity of 95 to 97%. For quality control, the chromatograph readings for organochlorine pesticides and their isomers were adjusted to follow the calibration of a 5-point curve from a linear regression. Reference samples were used to prepare the calibration curve using a ChemStation HP 3398A equipment, (ChemService, Inc., West Chester, Pennsylvania 19381, USA). In order to guarantee a recovery of 93%, fortification tests were carried out at different concentrations.

Concentration of lindane and endosulfan sulfate in oyster samples was performed following Murphy's technique (1972) [18], and modified by Waliszewski *et al*. (2008) [17]. The process started by weighing 10 g of lyophilized and milled sample for each season and placed in a Teflon beaker with 20 ml of acetone and 20 ml of hexane as solvents. The volume of this solution was divided into two parts; one was used for lipid extraction and the other to determine the concentration of organochlorine pesticides. The valves are placed on the lid of the vessel prior to incorporation of the sample and solvents, the lid must be gently adjusted to the bottom, the closed vessels are placed in the holder with a torque meter calibrated at 60 pounds of pressure, until the adjustment of the vessel in the same is achieved. Lipid extraction is performed by the modified pesticide method Green Chem of Murphy (1972) [18]. The temperature of the microwave oven CEM Model MARS-X was programmed at 110 °C and a pressure of 200 psi, with 10 minutes of maintenance and 10 minutes of cooling. After the process, they were placed under a fume hood for the control of toxic vapors. While there, the beakers with the samples were opened, then 15 ml of hexane and 15 ml of acetone were added and filtered through sterile Whatman No.4 filter paper. The filtered volume is placed in 250 ml flat bottom flasks and immediately a further washing of the material contained in the filter paper was performed, adding an additional 15 ml of hexane and 15 ml of acetone. The extracts collected in the flasks were allowed to cool for 15 minutes. Each flask was then placed in a roto-evaporator at a water bath temperature of 45 °C and 150 revolutions, until a 40 ml evaporate was obtained. After obtaining 40 ml, samples were allowed to stand and cooled for 30 minutes. Subsequently the residual material was weighed on a digital scale to determine gravimetrically the total lipids in each sample.

To continue the analysis of the pesticides lindane and endosulfan sulfate in oysters, 10 ml of the original solution was placed into a 50 ml tube with Bakelite stopper. Then, 1 ml of concentrated sulfuric acid was slowly added, not exceeding 4ml, and agitated vigorously for 1 min to precipitate the fat, after which the solution was left to rest for 15 minutes to separate phases. The supernatant was filtered in a funnel with No.4 filter paper onto a 8.0g layer of anhydrous sodium sulfate previously activated at a temperature of 100 °C. The filtrate obtained was placed in a 50 ml flat bottom flask, where 10 ml of ethyl ether was added to wash the sulphate and extract the remains of fat.

The sample was again placed in a roto-evaporator until approximately 1 ml of purified sample was obtained. The resulting extract was transferred via a Pasteur micro-pipette into a vial (Reacti-vial, Pierce®). Such vial and its respective replicas were placed in a glass bottle to protect it and kept in the freezer at 4 °C until its reading. Concentration of the organochlorine pesticides lindane and endosulfan sulfate in *C. virginica* oyster was performed with a gas chromatograph (Thermo Electron Model Trace GC Ultra 115V, Thermo Fisher Scientific Inc©, Monterrey, Nuevo León, México) with an Electron capture detector. Pesticide separation was performed on a 30m x 0.32mm x 0.25µm chromatographic column with 14% cyanopropylphenyl polysiloxane (Thermo Fisher Scientific Inc© Belleford, PA, USA). Where ultrapure nitrogen (Praxair-Mexico) was used as the entrainment gas at a flow rate of 2.5 ml/min. Operating temperatures were as follow: detector 300 °C, injector 250 °C and column 160 at 280 °C (4 °C/min). The injection volume was 1 µl in splitless mode.

III. RESULTS AND DISCUSSION

Mean concentrations of pesticides for each isomer, in ostion banks analyzed at the Mandinga (M) and Alvarado (A) lagoon systems located in the central Gulf of Mexico, are shown in Table 1.

Table.1. Pesticide isomers' mean concentration in ng·g^{-1} in oyster banks of the Mandinga (M) and Alvarado (A) lagoon systems.

Oyster banks	Lindane	Endosulfan sulfate
M1	7.88 ± 4.04	24.68 ± 1.20
M2	7.89 ± 4.02	11.38 ± 5.58
M3	8.05 ± 4.06	14.88 ± 11.50
M4	8.69 ± 5.15	19.32 ± 1.41
A1	4.11 ± 3.83	12.29 ± 2.84

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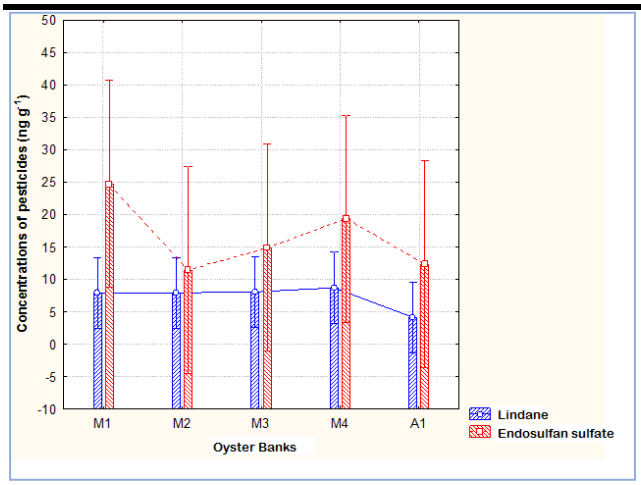

Fig. 2: Mean concentrations of the organochlorine pesticides lindane and endosulfan sulfate in oyster banks of the Mandinga (M) and Alvarado (A) lagoons.

Fig. 2, shows that mean concentrations of lindane in *C. virginica* oysters from the Mandinga lagoon do not show significant difference between banks and seasons, whereas it occurs in oysters from the Alvarado lagoon system. On the other hand, endosulfan sulfate fluctuates significantly between banks and seasons, presenting lower average concentration in the Mandinga bank 2.

The analysis of principal components by multiple factorial with respect to the contribution of the variables showed significant differences between endosulfan sulfate and lindane, which were notorious when they positioned at opposite poles of the axes (Figure 3).

Quantitatively, figures 4 and 5 show that the maximum concentration of lindane and endosulfan sulfate found in *C. virginica* oysters was during the north winds season. Mandinga bank 4, reports 12.62 $ng \cdot g^{-1}$ lindane and 39.35 $ng \cdot g^{-1}$ endosulfan sulfate, it was probably due to the disturbance caused by the wind in the sediments and the marine currents of the estuary which are pushed towards inland [19]. *C. virginica* oysters from Alvarado presented their highest concentration of lindane 7.60 $ng \cdot g^{-1}$ in the dry season, however during the north winds season no concentrations of this pesticide were found in *C. virginica* samples.

Lindane is not found naturally in the environment. The entry of lindane into the environment occurs during its formulation and its use as a pesticide. Bioconcentration in microorganisms, invertebrates, fish, birds and in humans takes place rapidly. Biotransformation and elimination also occur fast when exposure is eliminated, according to WHO (1991) [20]. Lindane is not toxic to bacteria, algae and protozoa [8], but is highly toxic to some fish and aquatic invertebrates. It has been detected in different mammals and birds in the Arctic [8]. Even at lower concentrations it has significant effects, due to this, they were related to the order of biological organization; for example from major to minor: effect on physiological activity> functional and structural alterations of molecules> cellular toxicity> mortality of organisms [21].

The presence of lindane has been detected in surface and drinking water, as well as in industrial and domestic effluents from Europe and the United States. Lindane was found in rainwater in Tokyo (29 - 398 ng/l) and soils in Ukraine contained lindane levels of 0.1-5 mg/kg [20] (WHO, 1991). On the other hand, in the lake Ologe in Nigeria, lindane was predominant in fish samples during rainy season [22].

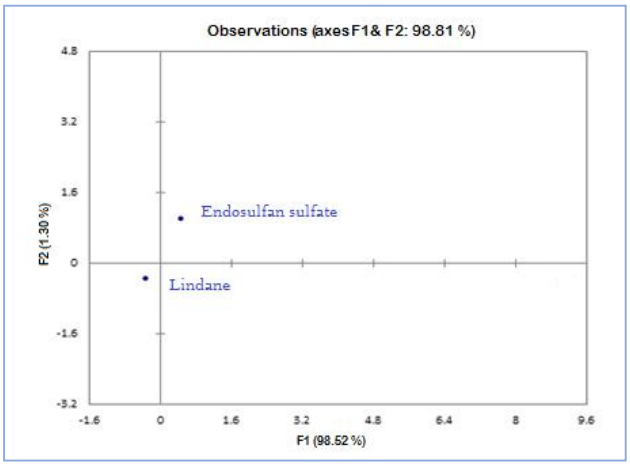

Fig. 3: Location of organochlorine pesticides, lindane and endosulfan sulfate in the axes.

Endosulfan sulfate has been classified by the United States Environmental Protection Agency (US EPA, 2002) [23] as highly toxic for marine and freshwater fish. Values of US EPA (2006) [23] for the protection of aquatic life are 0.22 $\mu g \cdot L^{-1}$. Crustaceans are particularly sensitive to endosulfan. It is known that this pesticide is highly toxic to many lobster and shrimp species, with average lethal concentrations (LC_{50}) close to 1 $\mu g \cdot L^{-1}$ [24].

Molluscs have been selected as monitoring species for their sessile and sedimentary lifestyle, and their ability to integrate environmental contamination in time and space [25]. For this ability to accumulate contaminants *C. virginica* has been used to conduct research to determine the concentration of organochlorine pesticides as endosulfan isomers and its metabolites in organisms from various lagoon systems where this bivalve lives. However, although there are precedents of a gradual increase in the concentration of this pesticide, there is a lack of consecutive monitoring in these lagoon systems. Likewise, Liu *et al.*, (2010) [26] indicated that few data are available to compare due to the scarce information on the concentration of endosulfan in fish and mollusc species.

In compiled investigations on the presence of endosulfan in *C. virginica*, it has been noted that the reported concentrations for β-endosulfan were higher than the

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concentration reported in various investigations compared to the alpha isomer and endosulfan sulfate [27]. This is in agreement with Berntssen *et al*. (2008) [28] about the relatively high concentrations of β-endosulfan, contributing to affirm that this isomer is more persistent. In contrast Wang *et al*., (2014) [29] indicated that biota such as fish and cultures can absorb α-endosulfan much easier than β-endosulfan, they also noted that in these samples the highest concentrations obtained were endosulfan sulfate followed by α and β endosulfan.

In many cases they are also difficult to remove by organisms because they are poorly soluble in water and tend to accumulate in fatty tissues. When organisms are eaten by others, then lindane accumulates in greater proportions in the final stages of the trophic chain [30,23]. So, a pesticide that is in very low concentrations in a forest or lake, without showing any risk, ends up being in concentrations tens or hundreds of times higher in the fatty tissues of the organisms like birds of prey and predatory fish or mammals that are located at the top of the trophic chain [31].

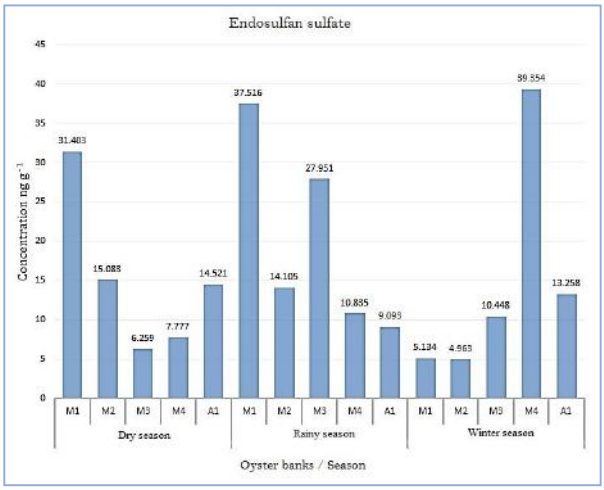

Fig. 4: Concentration of endosulfan sulfate during sampling periods, in the Mandinga and Alvarado lagoon systems, in Veracruz, Mexico.

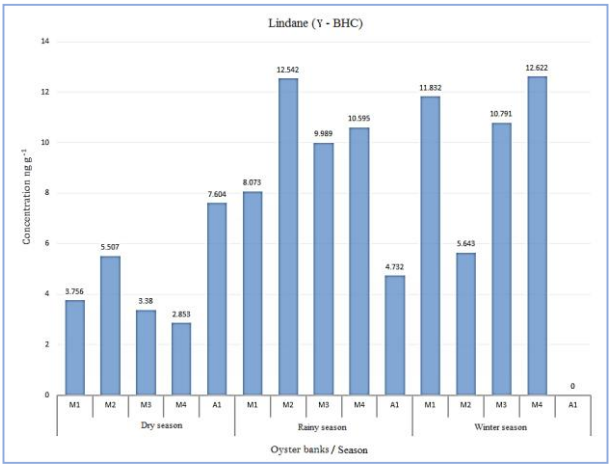

Fig. 5: Lindane concentration during sampling periods, in the Mandinga and Alvarado lagoon systems, Veracruz, Mexico.

IV. CONCLUSION

Organisms sampling was carried out in 2010. A year marked by an important meteorological event with maximum sustained winds of 190 km/h with gusts of 235 km/h, leaving rains in high zones registering maximums of 210 to 355 mm [32] that caused strong currents in the Jamapa river basin. Hence the *C. virginica* oyster of the Mandinga Lagoon System presented evidence of contamination with pesticides: endosulfan sulfate and lindane. At sampling point 4, endosulfan sulfate and lindane were recorded at concentrations that exceed the sediment quality criteria, studies report that these concentrations cause damage to benthic organisms. The concentration of the pesticides endosulfan sulfate and lindane registered in the Mandinga lagoon system is at levels that represent a risk to the benthic community and a potential risk for human consumption.

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