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Article

Hexachlorocyclohexanes, Cyclodiene, Methoxychlor and Heptachlor in Sediment of the Alvarado Lagoon System in Veracruz, Mexico

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Abstract: The objective of the research was to evaluate the concentration of organochlorine pesticides in sediment of the Alvarado Veracruz lagoon system, Mexico. To define the risk that causes to the public health the consumption of organisms that inhabit in sediments of this ecosystem. In 20 out of 41 stations analyzed, 11 prohibited organochlorine pesticides were identified, such as hexachlorocyclohexane, lindane, aldrin, dieldrin, endrin, among others. The highest concentrations were: aldrin 46.05; β-HCH 42.11; α-HCH 38.44; γ-HCH (lindane) 34.20; δ-HCH 31.61; methoxychlor 29.40; heptachlor epoxide 25.70; heptachlor 24.11; dieldrin 22.13; endrin 21.23; and endrin aldehyde 12.40 ng g-1. Concentrations reported are prohibited in international standards. In Mexico it is necessary to establish permissible limits in the environmental legislation for this matrix. There is a strong need to demonstrate, with scientific studies, the level of concentration reported by impact of compounds widely used in agricultural-livestock activities.

Keywords: bioavailability; toxicity; public health; pesticides; aquatic ecosystems

1. Introduction

Persistent Organic Compounds (POPs) are a chemical group that poses a high risk to public health and the environment, these compounds due to their transport capacity in the air or by run-offs reach areas far from where they were initially applied [1]. Because of their chemical characteristics, these organic molecules are included as prohibited in the Stockholm Convention list. Also, compounds such as polychlorinated biphenyls (PCBs), dioxins, furans, and a variety of organochlorine pesticides including aldrin, dieldrin, DDT, endrin, chlordane, hexachlorobenzene, mirex, toxaphene and heptachlor are prohibited [2,3]. Recent studies report the presence of these pesticides at high levels of concentration in soils, sediments, aquatic and terrestrial biota [4]. For its transport capacity, the presence of these compounds has been determined in various aquatic ecosystems of Mexico, becoming a risk to public health and the environment [5-8]. They have been found mainly among the coastal ecosystems located in the southeast region of the Gulf of Mexico. In addition, the presence of prohibited POPs as organochlorine pesticides: DDT, and their metabolites, endosulfan, lindane, among the main ones continues to be reported [9-13]. The main routes of entry of organochlorine pesticides correspond to those used in agricultural areas. These pesticides reach the coastal environment through rivers, drains, runoffs, and through atmospheric transport. Likewise, those that are used in health campaigns join them [14,15]. Once these compounds are found in aquatic environments, they are adsorbed by the organic matter particles in the water column. Here, several processes occur that cause the deposition of organochlorine compounds in the bottom of these bodies, where they are in direct contact with the bentonic organisms that inhabit this zone and are of commercial interest for human consumption [16, 17].

The sediment analysis in an aquatic ecosystem allows an integral estimation of its composition, since the sediment is the most stable matrix for the materials and substances dispersed in the water

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column. They are the main receptors of most of the contaminants deposited, due to their precipitation and accumulation capacity [17-19].

In coastal lagoons, it must be considered that chemical compounds undergo transformations in addition to precipitation; such as dilution, flocculation, sedimentation and degradation before finally reaching the sea [20]. In relation to the above, indicated that the sediment acts as a secondary source of pollution in the marine environment. The processes of transformation can cause a greater toxicity, as well as increase its residence time in the water column and its toxicity in the bentonic organisms that inhabit these ecosystems [11,15,16,21].

Sediments as a habitat for benthic biota are a source and mechanism for the removal of some pollutants, towards the streams and from the same aquatic stream. Therefore, sediments constitute a transport route for contaminants [21].

An efficient way to evaluate the presence of hydrophobic compounds in aquatic bodies is the analysis of contaminants in sediments and aquatic benthic biota, which also have a low solubility in water, a high solubility in lipids and a strong tendency to absorb organic material in soil and sediment [17,21]. Accordance to the above, it can be pointed out that chemical contaminants accumulating in bentonic organisms that inhabit sediments can be transferred to higher trophic levels through the food chain and thus constitute elements of risk to public health [11,13]. Therefore it is necessary to know the environmental quality of the sediments due to the effect it has on different trophic levels and on the transport of pollutants in the coastal zone. The objective of this work was to determine, in the sediment matrix of the Alvarado Veracruz lagoon system, the concentration of organochlorine pesticides; hexachlorocyclohexanes, endrin, methoxychlor and heptachlor, as elements of risk to the public health due to its consumption.

2. Materials and Methods

2.1. Study area

The Alvarado lagoon system is located in the southeast region of the state of Veracruz at coordinates 18°44'00 and 18°52'15 latitude North, 95°44'00 and 95°57'00 longitude West, located at 70 km from the southeast of the port of Veracruz with an elevation of 10 m above sea level [22-25]. This lagoon body has a total extension of approximately 6 200 ha and a maximum width of 4.5 m. The lagoon-estuarine system is made up of more than 100 brackish coastal lagoons and seasonally flooded areas, among the main lagoon bodies are: Alvarado, Camaronera and Buen País, and internal lagoons such as Pajarillos, Popuyeca, Embarcadero [26,27]. The main contributions of water to the system are the Papaloapan, Acula, Blanco and Limón rivers [22].

The average annual temperature is 26 °C and the coldest monthly average is above 18 °C. The dry season is from January to May, the rainy season from June to October and the north winds season is from November to January. The Alvarado lagoon is a water system with a high degree of turbidity, showing low transparency during the rainy season [22,25,28].

2.2. Collection and treatment of sediment samples for analysis

For sample collection, 41 sampling stations were geo-referenced in the study area (Figure 1). The sediment collection was performed in each season in a timely manner in triplicate during the dry season corresponding to the months of April, May and June.

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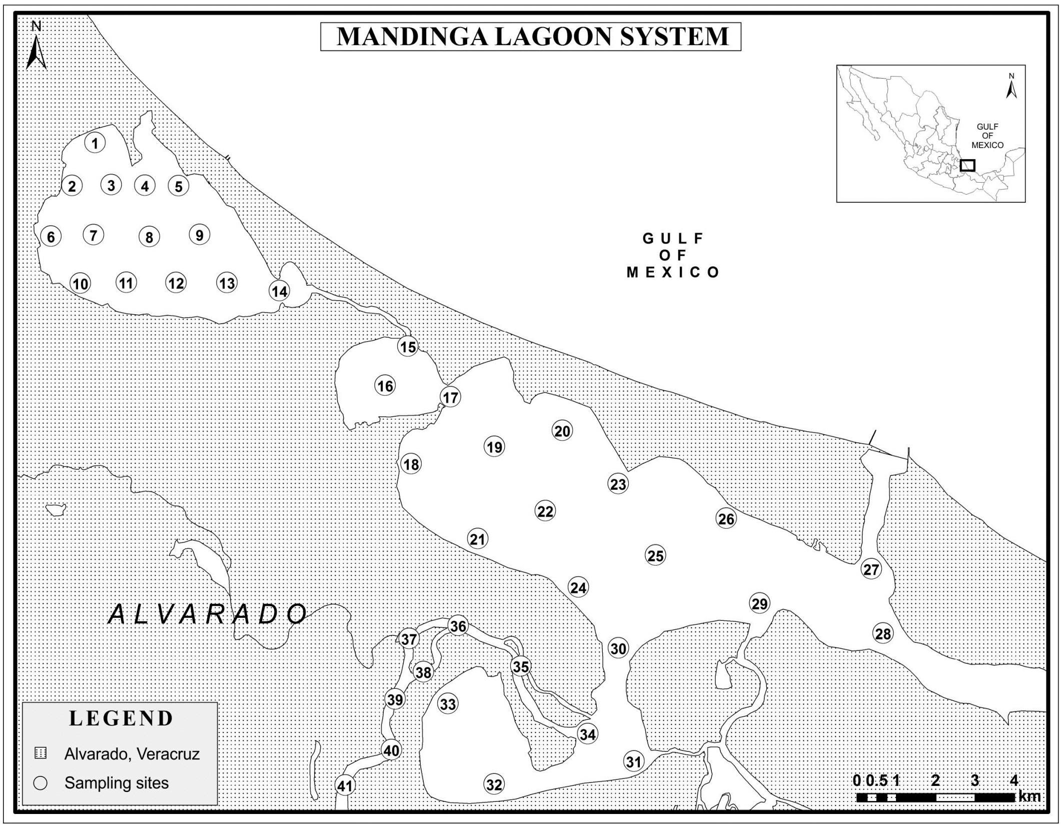

Figure 1. Sampling stations in the Alvarado lagoon system, Veracruz, Mexico.

Samples were collected at the stations using a Van Veen crab-type dredge and stored in Ziploc® polyethylene bags for storage at temperatures between 4 to 5 °C, while being transported to the laboratory, where they were frozen until treated and analyzed. The already frozen sediment samples were lyophilized in a Thermo Savant ModulyOD-114 kit for 72 hours at -49 °C and a vacuum pressure of 36x10-3 mbar. After lyophilized they were stored in sealed bags to be ground in a porcelain pistil mortar until obtaining a fine particle size. Finally, they were homogenized in a No. 30 sieve with an aperture of 595 μm. Samples were stored in a desiccator to avoid contact with the ambient humidity.

2.3. Laboratory analysis of fine sediment samples

The material used in the development of this research was prepared according to the pesticide residue analysis protocol standardized [29]. The material was washed with phosphate-free neutral Extran® soap for 24h, then rinsed with potable water, followed by distilled water (Milli-Q), finally washed with ethyl ether and acetone. To avoid cross-contamination of the samples, the purity of the ethyl ether used to wash the glassware was periodically evaluated, using gas chromatography.

2.4. Microwave extraction

Extraction of pesticides from the sediment was performed with the CEM microwave equipment MARS 5 (CEM Corporation, Matthews NC). Solvents and reagents used in all analyzes were reactive grade. The following were used: hexane (Backer) with a boiling temperature range of 40-50 °C; sulfuric acid (Merck) from 95 to 97% purity and sodium sulfate powder (Backer) previously activated and purified in a forced air oven model Riossa CF-102 at 650 °C for 24 hours.

The technique of Murphy [30] modified by Waliszewki [29] was used for the determination of the concentration of organochlorine pesticides. Using10 g of lyophilized and ground sample, placed then in a Teflon vessel with 20 ml of acetone and 20 ml of hexane as solvents. The microwave assisted extraction was carried out for a period of 20 minutes at 110 °C and a pressure of 200 PSI.

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2.5. Separation and cleaning of organochlorine pesticides in samples.

The sample was collected from the microwave extraction was placed in 250 ml flasks, this was cooled for 30 min and then this solution was filtered over a layer of sodium sulfate (8.0 g) and washed with 10 ml of petroleum ether. Subsequently, final purification of the sample was performed with a Florisil® cartridge as an adsorbent for 60-100 mesh chromatography (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Finally, the obtained extract was concentrated with a rotoevaporator to a volume of 1 ml of purified sample at 45 °C and stored in an amber vial (Reacti-vial, Pierce®).

2.6. Preparation of the calibration curve

Quality control of the readings was performed on the chromatograph for each organochlorine pesticide analyzed. These were a total of 11 pesticides: α-HCH, Lindane (γ-HCH), β-HCH, Delta-HCH (δ-HCH), Heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, endrin aldehyde and methoxiclor. It was performed by producing a linear regression of 5 points of the calibration curve. The reference standards and reference certificates used to make the calibration curve were ChemService, Inc., West Chester, Pennsylvania 19381, USA, a fortification test was also performed to ensure a recovery of 93%.

2.7. Quantification of organochlorine pesticides

The equipment used for the quantification was a Thermo Electron Model Trace GC Ultra 115V gas chromatograph (Thermo Fisher Scientific Inc ©) with electron capture detector. Pesticide separation was performed on a 30m x 0.32mm x 0.25μm of 14% cyanopropylphenyl polysiloxane chromatographic column from Thermo Scientific (Belleford PA, EU). Ultrapure nitrogen (Praxair-Mexico) was used as the entrainment gas at a flow rate of 2.5 ml/min. The operating temperatures were as follows: detector 300 °C, injector 250 °C and column 160 at 280 °C (4 °C/min). The injection volume was 1 μl in splitless mode.

3. Results and Discussion

3.1. Sediments as a source of pollutant distribution

The concentration of organochlorine pesticides analyzed at the sampling stations from number 30 to number 41 had values below the detection limit of the equipment (0.01 ng g^{-1}). In contrast, there were higher concentrations in the 20 analyzed sites, which included stations 1 through 19 and 25 in the study area. This indicates that for the first points there is a low risk for the aquatic biota that is in direct contact with the sediment, particularly for the molluscs, these organisms act as a link between the trophic levels, which are also important species indicative of pollution [31].

The presence of a higher concentration of organochlorine pesticides in sediments with respect to the concentrations found in the analyzed water samples, which is why it is important to analyze the sediment matrix [32]. The presence of organochlorine compounds represents an environmental risk, since among the environmental consequences are their bioaccumulation in the food chain and the alteration of aquatic ecosystems in both fresh and coastal waters. Likewise, the pollutant effect of these compounds in productive activities such as fishing, due to their risk in public health and in the environment must be considered [7].

In coastal environments and according to their application, organochlorine pesticides must be closely related to what is established by Mexican legislation, since there are several prohibited and highly toxic compounds. It have been indicated that five pesticides (aldrin, dieldrin, endrin, mirex and chlordecone) have been banned in Mexico, five are not marketed and are banned (chlordane, lindane, DDT, sulfluramide and endosulfan) and finally six others have never been approved for use (heptachlor, HCB, toxaphene, pentachlorobenzene, α and β-HCH) [7]. However, despite being banned, the presence of these compounds indicates their regular use and are found in the environment as degradation products of the original compounds [7].

3.2 Hexachlorocyclohexane (HCHs)

Lindane was the only compound identified at station 22 at a concentration of 4,051 ng.g^{-1} and the same case was found for α-HCH at station 28 with an average concentration of 4,051 ng.g^{-1} (Figure 2). The remaining 17 stations had concentrations with minimum and maximum values of: α-HCH with 2.13 (station 17) and 38.44 (station 4); β-HCH with 2.56 (station 6) and 42.11 (station 2); lindane (γ-HCH) with 1.45 (station 15) and 34.20 (station 13) and δ-HCH with 1.67 (station 15) and 31.61 ng g^{-1} (station 14). High concentrations of HCH are related to their extensive use reported [8], according to the information issued by CICOPLAFEST that lindane (γ-HCH) constitutes approximately 10 to 15% of the product hexachlorocyclohexane. This is authorized in Mexico for the treatment of oats, barley, maize, sorghum and wheat seeds, for industrial use in pesticide formulating plants, for the control of some cattle pests such as mites and lice. While urban use is exclusively in health campaigns [8,33].

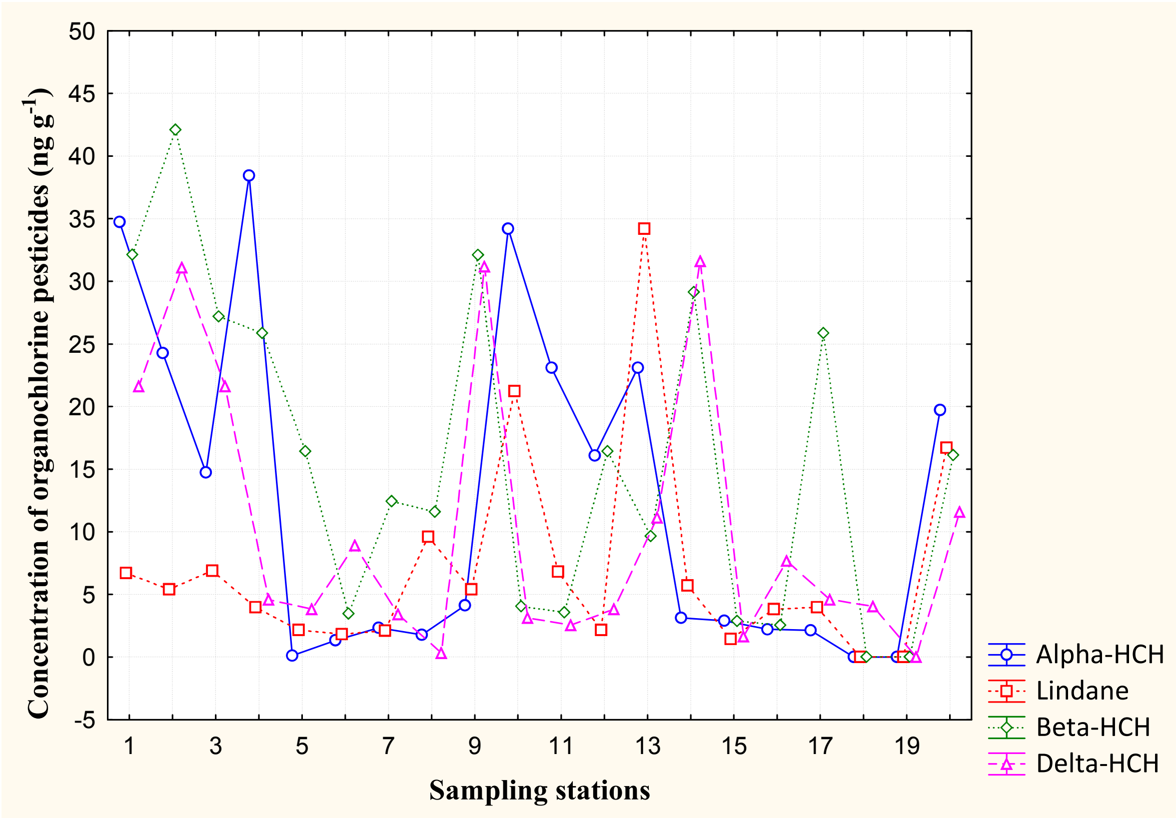

Figure 2. Concentrations of the hexachlorocyclohexane (HCHs) group in sediment from the Alvarado lagoon, Veracruz, Mexico.

The Agency for Toxic Substances and Disease Registry (ATSDR) also noted its extensive use as an insecticide in fruits, vegetables and forest plantations, and in animals [34]. In addition to storage spaces where these are maintained without a control of handling. The latter, in contrast to indicate that α and β-HCH have never been approved for use, in the case of lindane (γ-HCH) not marketed and found in prohibition process [7]. ATSDR has classified γ-HCH as a pesticide for restricted use, it can be used by licensed and certified persons for its control and management [34].

Concentrations reported of the HCH isomers in this research in the Alvarado lagoon system were lower than those found in the state of Ondo, Nigeria with concentrations of α-HCH (ND 8.07 ± 3.00); β-HCH (ND-10.91 ± 6.66 μg g^{-1}) and finally γ-HCH (ND-9.08 ± 0.02 μg g^{-1}) [32]. Also, it was reported in Densu River in Ghana, maximun sediment concentrations for γ-HCH of (0.555 ± 0.12 μg Kg^{-1}and 0.608 ± 0.24 μg Kg^{-1}), for δ-HCH (0.140 ± 0.05 and 0.14 ± 0.01 μg Kg^{-1}). Associating the variations in the distribution of the HCH isomers concentration with the use and handling of the

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mixture of this compound in different localities. Besides considering the greater contribution of γ-HCH compared to δ-HCH, due to its widespread use in the market as lindane (γ-HCH) [35].

Lindane (γ-HCH) even in low concentrations represents a risk to public health, this compound is listed as a carcinogen of Group 1 by the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) reported [36]. ATSDR (2016a) also noted that IARC has classified all HCH isomers as possible carcinogens in humans [34].

3.3. Cyclodienes (endrin and endrin aldehyde, aldrin and dieldrin)

The maximum concentration range of the cyclodiene family in the sediments of the Alvarado lagoon was as follows: aldrin 46.05; endrin 21.23; endrin aldehyde 12.40 and dieldrin 22.13 ng g-1 (Table 1, Figure 3). The previous concentrations were higher than those reported in Navachiste-Macapule lagoon, Sinaloa, Mexico. Where a maximum mean concentration of aldrin 0.58 ng g-1; endrin 4.93 ± 5.82 ng g-1; endrín aldehyde 0.76 ± 0.39 and dieldrin 1.13 ± 0.80 ng g-1 were obtained [37].

Table 1. Concentration of organochlorine pesticides (ng g-1) in sediment of the Alvarado lagoon system in Veracruz, Mexico.

Compound	Minimum	Maximum	Mean	Standard deviation
α-HCH	0.121	38.44	12.428	13.264
γ-HCH (Lindane)	1.45	34.20	7.010	8.287
β-HCH	2.56	42.11	15.683	12.737
δ-HCH	0.34	31.61	10.424	10.801
Heptachlor	1.98	24.11	8.621	8.000
Aldrin	2.11	46.05	11.667	12.088
Heptachlor epoxide	2.13	25.70	7.052	5.884
Dieldrin	2.18	22.13	9.041	6.051
Endrin	0.34	21.23	8.420	7.724
Endrin aldehyde	0.01*	12.40	2.581	3.224
Methoxichlor	1.13	29.40	5.650	6.561

*Limit of detection of 0.01 ng g^{-1} (< LDD).

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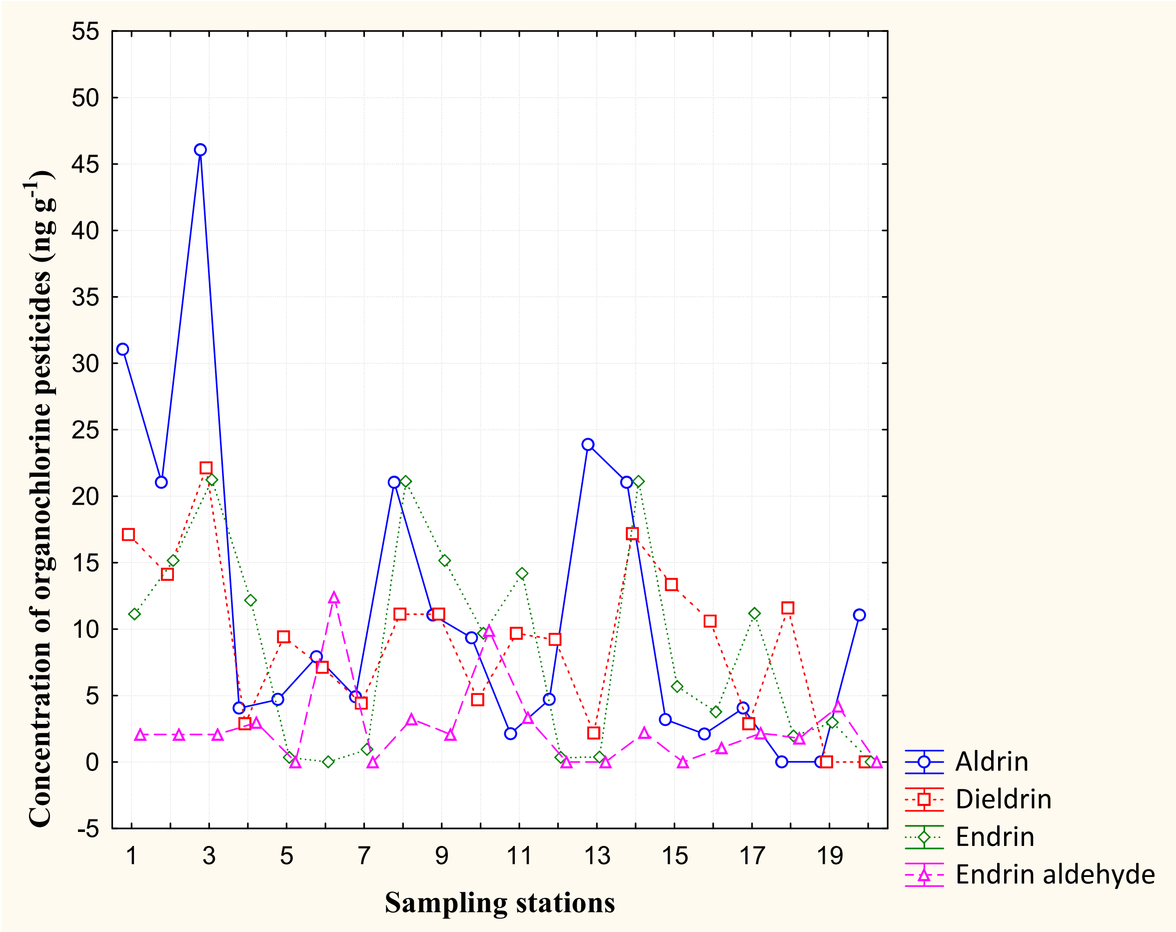

Figure 3. Concentrations of the cyclodiene family in sediment of the Alvarado lagoon in Veracruz, Mexico.

The high concentrations of endrin, followed by dieldrin, endrin aldehyde and finally aldrin indicate a recent application. In addition, these maximum concentrations can be associated with the rainy season due to runoff from the agricultural fields [37].

In the case of Ondo state reported concentrations of these compounds higher than those reported for Navachiste lagoon, these are: aldrin (ND; 6.55 ± 0.02 $\mu g.g^{-1}$); dieldrin (0.01 ± 0.01, 7.62 ± 5.72 $\mu g.g^{-1}$) and endrin (ND-21.28 ± 3.17 $\mu g.g^{-1}$) [32]. Furthermore, reported on benthic sediment from Agboyi Creek, Lagos, Nigeria, also high concentrations of aldrin (43.6 ± 2.1 ng g-1); endrin (8139.5 ± 2.3); endrin aldehyde (536.2 ± 7.4) and dieldrin (38.9 ± 7.5 ng g^{-1}) [38].

Despite variations in concentrations among various investigations, the detection of endrin compounds suggests its continued use, despite its prohibition by government laws [37]. In Mexico, however, only the Comisión Federal para la Protección contra Riesgos Sanitarios (COFEPRIS) is the federal agency that issues this type of recommendations on the use of pesticides. ATSDR issued a recommendation for lifetime water consumption for children and adults with endrin of 0.002 mg L^{-1} and in the case of ambient waters recommended a maximum level of 0.001 mg L^{-1} to protect the health of human beings [39].

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3.4. Methoxichlor and heptachlor

The maximum value of methoxychlor obtained in sediment of the Alvarado lagoon was 29.40 at station 19 (Figure 4), followed by heptachlor with 24.1 (station 3) and heptachlor epoxide with 25.79 ng g^{-1} (station 11). These sediment concentrations are lower than those reported in Lagos, Nigeria with maximum concentrations of methoxychlor 146.7 ± 8.2, heptachlor 125.6 ± 8.5 and heptachlor epoxide 403.6 ± 9.3 ng g^{-1} [38]. The above concentrations for heptachlor are high, considering that ATSDR reported 0.01 mg L^{-1} as the limit for most crops. And in case of food, the limit reported in edible seafood was 0.3 mg L^{-1} [40].

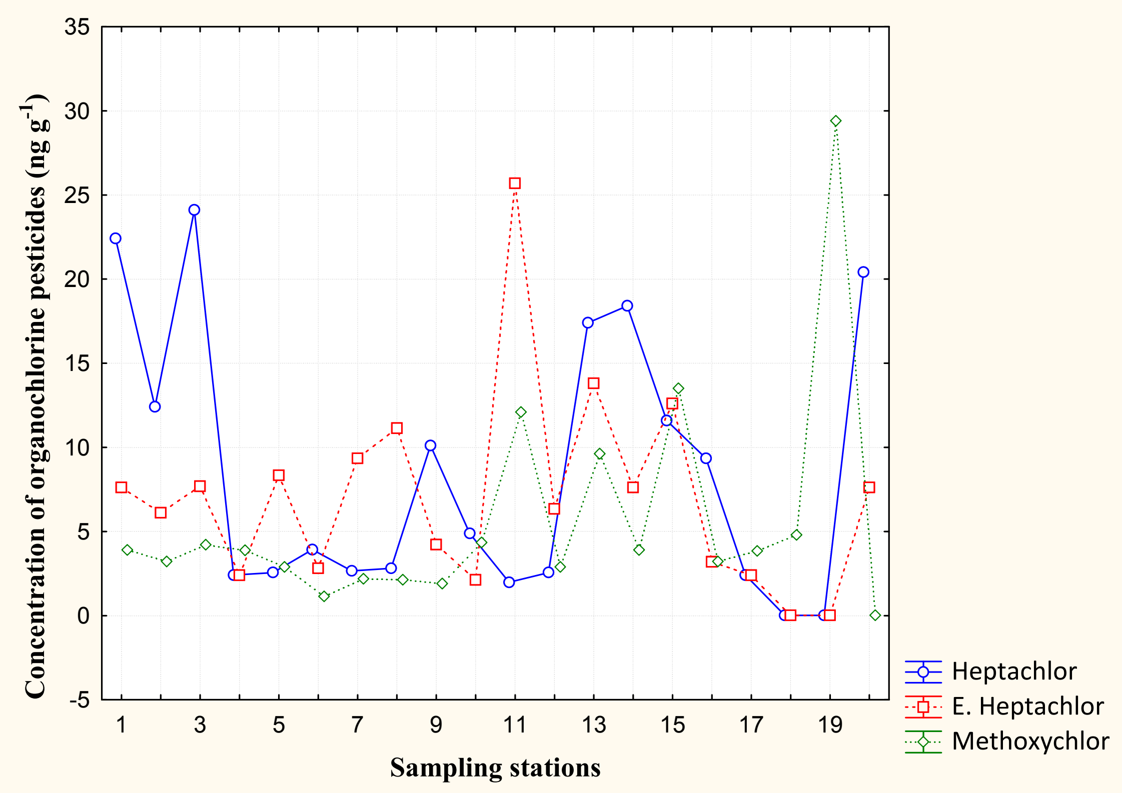

Figura 4. Concentrations of methoxichlor and heptachlor in sediment of the Alvarado lagoon in Veracruz, Mexico.

The detection of methoxychlor and heptachlor, as well as other organochlorines (endrin and DDT) suggests its continued use, despite their prohibition by governmental laws [37]. It must be considered in the case of methoxychlor that most of it enters the environment when applied to agricultural crops, forests and livestock. The degradation of this compound is slow in environmental matrices (air, water and soil), due to the effect of sunlight and microorganisms this degradation can take a period of several months. Also, some degradation products of methoxychlor may be as damaging as the parent compound. Among toxicological effects, the presence of high levels of this compound in animals produced tremors and convulsions, as well as affecting fertility [41].

A higher concentration of methoxychlor (12.70 ± 15.20 ng g^{-1}), compared to heptachlor with 5.95 ± 6.72 and heptachlor epoxide with 0.50 ± 0.14 ng g^{-1} [37]. They indicated that a high concentration of the first compound in the sediments suggests its constant use in the regions with influence in the lagoon system. The above according to the ATSDR is due to the fact that methoxychlor released into air is eventually deposited in soil, where it adheres firmly to particles. In addition to its zero solubility in water, this matrix is adhered to sediments and deposited in the bottom of bodies of water. It is important to mention that the amount of methoxychlor that may be present in drinking water is 0.04 mg L^{-1} [41].

The compounds heptachlor and heptachlor epoxide, like other organochlorines, including methoxychlor, adhere strongly to soil and sediment, evaporate slowly in the air and do not readily dissolve in water. The heptachlor epoxide dissolves readily in water opposite to heptachlor and evaporates slowly therefrom. Therefore, heptachlor epoxide degrades very slowly in the environment and can remain many years in soil and water [40].

In addition to its persistence in the environment, the accumulation in living beings and therefore their toxicity must be considered. ATSDR noted that heptachlor and heptachlor epoxide accumulate in fish and livestock, whereas in human beings, heptachlor epoxide can be stored in body fat. It has been shown that the latter can be detected in the body fat of a person 3 years after exposure to this substance. Also, heptachlor epoxide is more toxic than heptachlor in laboratory animals [40].

With respect to the long-term effect on health and due to the heptachlor toxicity and its metabolite, the International Agency for Research on Cancer (IARC) has classified both compounds as possible carcinogenic in humans [40].

4. Conclusions

Concentrations of organochlorine pesticides in the analyzed sampling stations show their influence on the dynamics of the Alvarado lagoon system. These compounds are exported to the coastal zone and finally deposited in open sea.

This research highlights the need for constant monitoring in sediments of the lagoon system and hydrological basins of influence that are located in different regions of Mexico. Sediments are the habitat of different aquatic organisms, such as crustaceans and bivalve molluscs that get contaminated and therefore, when consumed by people could represent an element of risk to public health.

Compounds highly toxic to public health of the group of organochlorine pesticides, such as Hexachlorocyclohexanes, cyclodiene, methoxychlor and heptachlor were identified in this study. Concentrations found indicate their illegal use in Mexico, despite being banned internationally.

Efficient regulation is required in the use and management of insecticides. As well as promoting the use of environmentally friendly compounds in agricultural and livestock activities.

It is necessary to reflect on including the sediment matrix in the environmental legislation of Mexico, in order to establish the maximum permissible limits in aquatic ecosystems. Considering that these sediments are the habitat of important species of aquatic organisms of national and international consumption.

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