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Exposure Assessment to Persistent Organic Pollutants in Wildlife: The Case Study of Coatzacoalcos, Veracruz, Mexico

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1. Introduction

Until the early 70s, it was thought that pollution was a phenomenon circumscribed to zones where pollutants were generated. Because of that, in each country concern was limited to regions where pollutant concentration was higher or its danger was greater. However, it has gradually become aware that pollution is a problem that affects everybody and, because of that, everybody is responsible to control it, regardless of the sites distance where pollutants are produced. Therefore, the problem of pollution has become a global phenomenon. Mankind has always depended on natural resources located in the region where they dwell. Nevertheless, the fast population growth coupled with a fast agricultural and industrial development as well as life style changes have increased emissions of pollutants in different ecosystems.

Persistent organic pollutants (POPs) is a group of compounds chemically very stable, able to travel considerable distances and it is resistant to natural degradation processes, most of them were produced to be used as pesticides and certain chemicals to be used as industrial processes, and others are generated as by-products unintentionally from human activities, such as combustion processes or power generation (PNUMA, 2005). Most of these compounds are highly toxic; they bioaccumulate in human and animal tissue, mainly in the fatty tissues, and can damage different organs and systemic targets such as the liver, kidney, hormonal system, nervous system, etc., of both humans and wildlife. According to the Stockholm Convention held in 2001, there are twelve compounds known as POPs: pesticides (DDT, aldrin, chlordane, dieldrin, endrin, mirex, toxaphene and heptachlor), industrial chemicals (hexachlorobenzene and polychlorinated biphenyls -PCB-) and unintentional compounds (dioxins, furans, PCDD-and PCDF-) [Albert, 2004]. In May of 2009 nine new Chemicals were added to the POPs list: alpha hexachlorocyclohexane, beta hexachlorocyclohexane; hexabromodiphenyl heptabromodiphenyl ether and ether tetrabromodiphenyl pentabromodiphenyl ether and ether chlordecone, hexabromobiphenyl, lindane, pentachlorobenzene, perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride.

POPs main route of entry into the organism is food. However, we cannot ignore environmental exposure (inhalation) and dermal exposure (accidents). Because of their properties, POPs are classed as persistent, bioaccumulative and toxic. Therefore, POPs are to be considered as one of the most harmful groups of Toxic environmental pollutants to humans and wildlife. In countries where these compounds have been used are frequently found residuals in food. They are a problem because of their persistence in the environment and characteristics of bioaccumulation and biomagnification along the food chain and because these compounds generate toxic effects in both human population and in biota.

POPs, mainly organochlorine compounds make up a big part of hazardous waste. Mexico annually generates approximately 8 million tons of hazardous waste. Of this amount, only 12 percent is handled properly. The question is: Where does the remaining 88% go? To make matters worse, Mexico's infrastructure for hazardous waste is by far insufficient.

Coatzacoalcos, Veracruz is one of the most commercial and industrialized ports in Mexico. Presently, the Coatzacoalcos River and the areas surrounding it are regarded by many as some of the most heavily polluted sites in Mexico. Several of Mexico's chief petrochemical complexes, such as Cangrejera, Morelos and Pajaritos are based in the region. Furthermore, there have been various toxic substances present in the area which have been stored inside environmental and biological compartments, including persistent organic pollutants (Espinosa-Reyes et al., 2010; Gonzalez-Mille et al., 2010; Stringer et al., 2001), polycyclic aromatic hydrocarbons, volatile organic compounds (Riojas-Rodriguez et al., 2008), polybrominated compounds (Blake 2005), dioxins and metals (Petrlink & DiGangi 2005; Rosales 2005; Vázquez-Botello 2004).

A number of POPs have been registered in Coatzacoalcos. One of these, Hexachlorocyclohexane (HCH), is a manufactured chemical from which there are, theoretically, eight chemical forms or isomers. The three most common isomers are α-HCH, β-HCH and γ-HCH (commonly called lindane). Lindane is used as a pesticide on fruit and vegetable crops as well as on forest plantations; it is also found in medications to treat diseases such as scabies and pediculosis. There are no records indicating that lindane has ever been manufactured in Mexico; however, approximately 20 tons of these compounds are imported and subsequently used in Mexico each year. At present, lindane is authorized for use in Mexico for ectoparasite control in livestock for ticks, fleas, and common fly larvae. It is also registered for use as a seed treatment for oats, barley, beans, corn, sorghum and wheat. Pharmaceutical uses of lindane in Mexico include the formulation of creams and shampoos for scabies and lice treatment (CEC, 2006; ATSDR, 2005). In 1994, the Canadian Environmental Protection Act (CEPA) proclaimed hexachlorobenzene (HCB) as highly toxic. HCB is a manufactured chemical (which was) used as a wood preservative, as a fungicide for treating seeds and as intermediary in organic syntheses. Additionally, hexachlorobenzene can be formed as an unwanted byproduct in preparation processes like in the synthesis of organochlorines from high-temperature sources (Sala et al., 1999; Newhook & Meek 1994). Dichlorodiphenyldichloroethane (DDT) is a synthetic organochloride which is relatively stable with slow degradation rates through sunlight or oxidation, and possesses good absorption capacity and resistance to biodegradation in sediments and soils. It is also insoluble in water (CEC, 2001). In 1945, DDT was used for the first time in Mexico for the control of Malaria, and was widely used in agriculture between the 50s and 70s (CEC, 1997). The use of DDT in the Malaria Control Program was abandoned in the year 2000, when it was replaced by pyrethroids. Polychlorinated

biphenyls (PCBs) are allowed in “totally enclosed uses” such as coolants and lubricants, in transformers and capacitors. Dioxins are produced during the combustion of organic materials containing chlorine as well as during the manufacture of various chlorine-containing chemicals, such as ethylene dichloride. Existing involuntary sources of intake include electric arc furnaces, shredders, sinter plants, cement plants, cremation facilities, and coal-based power plants (Lutharddt et al., 2002).
Biomonitoring wildlife can be used to detect chemical pollution and to evaluate the ecosystem’s health, using test species as systematic models in the evaluation of risks associated to paths of real exposure. Wildlife species residing in polluted sites are exposed to complex mixtures of pollutants through multiple pathways which could hardly be evaluated in lab studies. The main purpose of this research was to pinpoint exposure levels to POPs in wildlife from different sets of ecosystems throughout the industrial area of Coatzacoalcos, Veracruz, Mexico to obtain a baseline of the ecological condition of this region.

2. Materials and methods

2.1 Test area, sampling sites and species selection

The Coatzacoalcos region is located in the South eastern State of Veracruz, Mexico, in the municipality under the same name, at 18° 8' 56 " N and 94° 24' 41" W. The average altitude is 14 m.a.s.l. The predominant climate is tropical rain [Am (i') gw"], the average annual temperature is 24.5ºC and average annual rainfall is 2780.1 mm (García, 2004). The main inland body of water is the Coatzacoalcos River, which has an area of 322 km. It originates above 2000 m in elevation in the State of Oaxaca and over its course is fed by countless other rivers (Jaltepec, Coachapa, Uxpanapa, Calzada) and streams (Teapa, Tepeyac, San Francisco) which inflows contribute to the discharge of pollutants (Páez-Osuna et al., 1986.; Rosales-Hoz & Carranza-Edwards, 1998). The region is comprised of urban, industrial, livestock, riparian and wetland areas. However, its main activity is chemical, namely petrochemical (Ruelas-Inzunza et al., 2007).
In October 2006, six sampling stations for biological sampling were set up at the lower basin of the Coatzacoalcos River (Fig. 1). The selection of sampling sites was based on wind direction, location of industrial zones and urban areas, the presence of organisms and the influence of riparian systems as well as on previous investigations within the area (Páez-Osuna et al., 1986; Rosales-Hoz & Carranza-Edwards 1998; Stringer et al., 2001; Bahena-Manjarrez et al., 2002.).
In this research we selected earthworms, crabs, fish, toads, turtles, iguanas, and crocodiles to measure levels of POPs in muscle or blood. These groups are critical species because they have an important role in the ecosystems dynamics and/or an importance (economic, cultural and scientific) for man. Species were selected according to the following criteria: The kind of pollutant located in the study area. Based on literature, a revision on the pollutant environmental behavior was conducted, considering their physiochemical characteristics as well as environmental parameters (humidity, temperature, pH, type of soil, etc.) that can influence in the environment pollutant levels. Once pollutant groups to evaluate were determined, potential pathways and exposure routes were established. An important criterion that was also considered when selecting animal groups was their biology, as it should be well documented; finally groups that are relatively easy to capture and handle were selected.

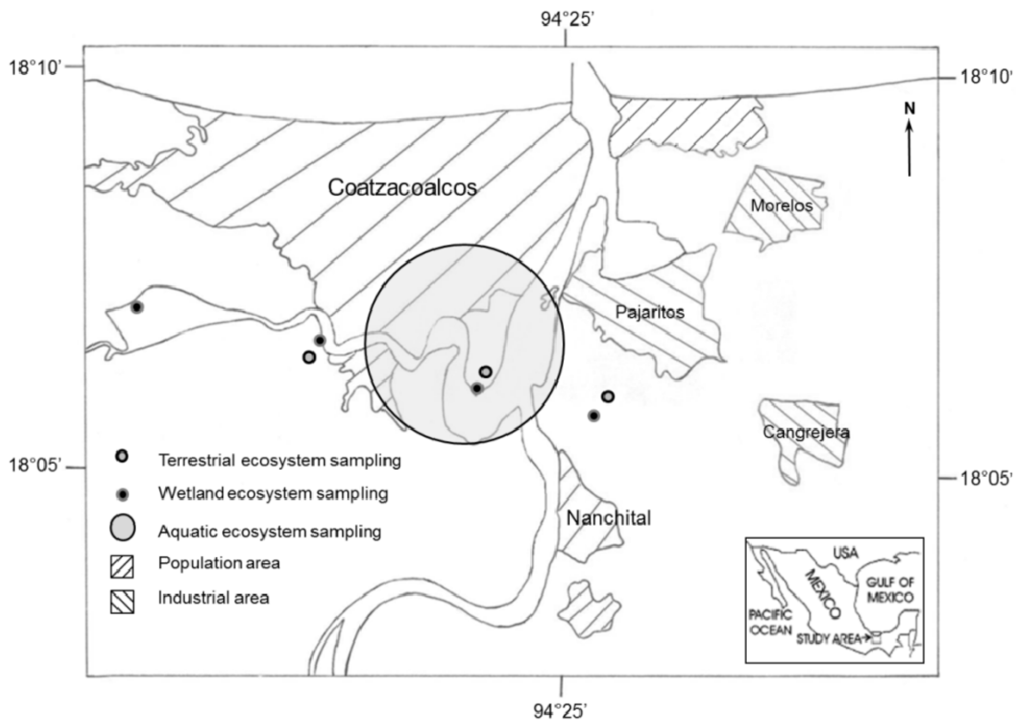

Fig. 1. Study area and location of sampling sites in the region of Coatzacoalcos, Ver.

2.2 Ecological importance of selected species

2.2.1 Earthworms

Earthworms' ecological importance is that they are decomposer organisms (important in biogeochemical cycles) and because of that, they have an important role when adding nutrients to soil (they favor the availability of nitrogen, phosphorus and sulfur) which can be used by vegetable species (Reines et al., 1998; Legall, 2006); they are also an important link in the trophic chain, mainly for some bird species and it has been proven that earthworms can accumulate important metal concentrations (Sánchez-Hernández, 2006). Earthworms can be found in many types of soil and are vulnerable to impacts occurred in soil, their small size represents an advantage to be handled; their distribution is ubiquitous in edaphic horizons with detritus, they are easy to capture, have a close contact with soil, and have a short life-cycle favoring the study of several generations (Ogunseitan, 2002). Persisting organic pollutants have the capacity to bioaccumulate and biomagnify along the trophic chain, as well as animals that belong to decomposers or detritivores levels are very important for the ecosystem functioning, therefore, if animals that are part of soil are affected by pollutants that may show in the ecosystems' health.

2.2.2 Crabs

Crabs are ubiquitous in all temperate and tropical regions in the world. In the wetland ecosystems they are an ecologically important kind, because they play a primary role in the decomposition of organic material and the addition of nutriment to soil. Due to the fact that they build up their galleries by the rivers' basin, lakes or seas, they spend part of their life on land surface and when tides rise they shelter in their burrows (≈ 30 cm. deep) under water. They mainly feed themselves with detritus, so they are excellent filtering organisms, capable to accumulate great pollutant quantities.

2.2.3 Iguanas

There are different kinds of iguanas, however the most common ones in the Coatzacoalcos region are the green iguana (*Iguana iguana*) and the black iguana (*Ctenosaura* spp.); Distribution of both goes from the south of Mexico to South America. According to Lara-Lopez and Gonzalez-Romero, (2002), iguanas are herbivores, the diet of the green iguana is composed mainly as follows: leaves (57.36%), flowers (24.15%) and fruits (3.43%), most of their life is spent on top of the trees, because of the previously said, this specie may be used as a POPs bio-monitor in air. In many coastal communities in Mexico, it is usual to eat iguana as an important source of protein, and it is believed that their blood and eggs contain a lot of energy and help to prevent certain diseases such as anemia. Furthermore people use the skin of this reptile and dissected to sell as ornaments (Alvarez del Toro, 1982). In southern Mexico (Leon & Montiel, 2008), in Central America (FAO, 1997) and in parts of South America, the iguana is one of the most consumed wildlife species. Iguanas have been an important source of protein for humans for over 7000 years FAO (1997). Many of the rural inhabitants of Central America still rely on iguana as a protein source; however, consumption of these species are not uniform during the year because in most cases eating frequency depends on the availability in certain times or seasons that are usually 3 to 4 months per year (Pers. Obs.).

2.2.4 Fish and invertebrates

Fish and aquatic invertebrates are commonly used to monitor pollutants because they bioaccumulate toxic substances and are wile spread, coupled with the diversity and importance of these environments. It has been observed that they are highly sensible to changes in the aquatic environment as well as to low concentrations of environmental pollutants, (Russo et al., 2004; Klobučar et al., 2010). On the other hand, fish have a crucial position in the toxicological field, due to the fact that they have been widely used in studies related to human and ecological health. Fish study includes a wide variety of approaches to detect aquatic pollution impacts from direct measures of mortality, to the analysis of demographical dynamics and the community structure, and to the detection of measures of sub cellular changes (Di Giulio & Hinton, 2008).
At the same time aquatic invertebrates and specifically crustaceans are organisms that have a wide distribution (example: marine, terrestrial and freshwater environment), they are organisms that are in close contact with pollutants in sediment, so they have been used in countless eco-toxicological studies. They have proven to be useful to evaluate effects on different pollutants. They have also served as aquatic pollution indicators. In addition, they may be a source or exposure for local consumers (Nacci et al., 1996; Rinderhagen et al., 2000; Rigonato et al., 2005; Külköylüolu 2004; Regoli et al., 2006). Otherwise, spatial distribution of pollutants on sediments and biota in aquatic ecosystems have been related to a great variety of biological answers in populations and fish/invertebrates communities, with the purpose to determine a possible relation between pollutants in the environment and health in the organisms (Adams et al., 1999).

2.2.5 Giant toads

The giant toad (*Rhinella marina*, after *Bufo marinus*) is a native and geographically widespread species in Mexico and Central America (Zug & Zug 1979). It is an omnivorous and opportunistic species (Zug & Zug 1979), which indicates that toads would integrate different exposure paths due to the ingestion of a wide variety of food items and amphibious living habits. The giant toad is one of the largest amphibians in Mexico (adult body length ranges from 10 to 17 cm), with a life expectancy from 10 to 15 years in the wild. The high lipid-somatic index (2 to 10% compared to less than 0.1% in most anuran species after the spawning period) and the elevated hepatosomatic index (Feder & Burggren 1992) along with its breeding biology make this species prone to bioaccumulation of organic and inorganic pollutants and their toxicological effects (Sparling et al., 2010; Linder et al., 2003). Recently, the giant toad has been used as an aquatic ecosystem biomonitor in the evaluation of air pollution (Dohm et al., 2008), infectious diseases (Zupanovic et al., 1998), organochlorine pesticides (Linzey et al., 2003) and endocrine disruptors (McCoy et al., 2008).

2.2.6 Turtles

Slider turtle (*Trachemys scripta*) is geographically widespread across Mexico and Central America (Burger and Gibbons, 1998). They are eligible species because they have several characteristics associated with their metabolism, life history and ecology (Overmann & Krajicek, 1995). These turtles are generally omnivorous and have temperature-dependent of sex determination and studies have investigated contaminant effects on this process (Selcer 2006), this makes them ideal for studies of chronic exposure of local pollutants. This species has been employed for exposure assessment to metals, radiation, organochloride pesticides and polybrominated biphenyls (Bergeron, et al., 1994; Bickham, et al., 1998; Burger and Gibbons, 1998; Lovelette & Wrigth, 1996; Meyers-Schöne & Walton 1994; Willingham et al., 1999; Willingham et al., 2000). In Mexico, slider turtles are considered an endangered species and are protected by Mexican laws.

2.2.7 Crocodiles

Swamp crocodiles (*Crocodylus moreletii*) are aquatic reptiles living in Mexico's tropical regions. They have a life strategy based on late maturation; they are extremely long-lived animals, show parental care and determine sex depending on temperature (Selcer, 2006). Crocodiles reach the highest levels of food chains, so they are useful for the evaluation of persistent and biomagnifying pollutants. Around the world, their populations are endangered (including Mexico), and because of that the concern on effects (mainly reproductive ones) of pollutants in their populations has increased (Guillette et al., 1999); it must be mentioned that the highest DDE levels registered in wild reptile were found in these organisms (De Solla, 2010). Different Crocodylia species have been used to evaluate heavy metals as well as persistent organic compounds.

2.3 Biological sampling techniques

Wild Earthworms (*Eisenia* sp) were collected by excavation. Crabs were harvested using pitfall traps (*Uca* sp) and traditional fishing gear (*Callinectes* sp). fish (*Aplodinotus* sp, *Ariopsis felis*, *Centropomus parallelus*, *Eucinostomus* sp, *Eugerres axillaris, Gobiomorus* sp, *Menticirrhus* sp, *Mugil cephalus* and *Oreochromis* sp) were caught using traditional fishing gear (i.e. cast net) with the help of fishermen. Giant toads (*Rhinella marina*) were collected from each site using nets in nocturnal transects within an area of 10,000 m^2. Crocodiles (*Cocodrylus moreletti*) and Iguanas (*Iguana iguana*) were caught using a noose trap. Turtles (*Trachemys scripta*) were captured with a baited piper trap placed near fallen trees and along the edge of the river during the afternoon and checked early the following morning.

Immediately after capture, organisms were measured, weighed and sorted by type of species; type of ecosystem (terrestrial, aquatic and wetland) and by feeding behaviours (carnivores, omnivores, detritivores and herbivores). Blood samples drawn were obtained using heparinized syringes on endangered animals (turtles and crocodiles). All organisms were subsequently released. Samples were stored at 4ºC for transport and subsequent laboratory analysis. Dissection was performed on each of the specimens from the rest of different species to extract the muscle tissue. The tissue was placed in amber glass containers and frozen at -20ºC until analysis. All organisms were collected with a Scientific Collector's Permit (Wild Fauna and Flora Scientific Collector) issued by México's SEMARNAT (Ministry of Environment) or Secretaría de Medioambiente y Recursos Naturales-No. FAUT-0133.

2.4 Analysis of blood and tissue residues

Concentrations of the following compounds were tested for on biological samples: α-, β-, γ-hexachlorocyclohexane (HCH), hexachlorobenzene (HCB), aldrin, dieldrin, mirex, α-, γ-chlordane, oxychlordane, trans-, cis-nonachlor, heptachlor epoxide, p, p'-DDT, p, p'-DDE, polychlorinated biphenyls (PCBs, IUPAC No 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 187, 180, 183, and 170) and polybrominated diphenyl ethers (PBDE, only on some species of fish). The method of extraction, separation and cleaning of muscle tissue was carried out according to the method established by Jensen et al., (2003) with slight modifications (Gonzalez-Mille et al., 2010) and Dallaire et al., (2006) for blood samples. The endrin-C13 and PCB 14-C13 were used as internal standards and were added to all samples. The chromatographic method (Gas chromatography–mass spectrometry GC-MS) was carried out according to that reported by Trejo-Acevedo et al., (2009). The detection limit for POPs was approximately 0.3 mg/L.

3. Results and discussion

3.1 Terrestrial ecosystem

In Table 1, it can be observed that earthworms have the highest concentrations of polychlorinated compounds biphenyls (PCBs) y persistent organic pollutants (POPs), followed by iguanas and finally by crabs. PCBs congeners that were analyzed were (PCBs 105, 128, 138, 153, 156, 170, 180 y 183).

ECOSYSTEM	SPECIES	α-HCH	β-HCH	γ-HCH	DDT	DDE	Mirex	ΣPCBs	ΣPOPs
TERRESTRIAL	Eisp	12.8	N.D.	106.3	2.5	13.2	N.D.	13.4	146.2
	(n=6)*	(5.8 - 31.4)		(39.4 - 196.0)	(2.5 - 2.5)	(0.3 - 57.2)		(2.3 - 39.2)	(50.3 - 323.8)
	Igig	4.76	0.59	0.55	N.D.	0.04	0.36	0.11	6.42
	(n=3)	(4.33 – 5.41)	(0.48 – 0.71)	(0.45 – 0.66)		(N.D. – 0.06)	(0.27 – 0.49)	(N.D. - 0.22)	(5.97 – 6.82)
	Ucsp	0.24	0.37	0.06	0.02	0.04	0.73	0.04	1.52
	(n=2)*	(0.17 – 0.31)	(N.D. – 0.74)	(0.05 – 0.08)	(0.020– 0.026)	(0.04 – 0.05)	(0.72 – 0.74)	(N.D. – 0.09)	(1.25 – 1.78)

Values represent the mean and range. Eisp: *Eisenia* sp., Igig: *Iguana iguana*, Ucsp: *Uca* sp., * Pool samples, N.D.: non detected

Table 1. Concentrations of persistent organic pollutants (ng/g tissue) from terrestrial wildlife collected in Coatzacoalcos, Veracruz.

Registered results of iguanas (*Iguana iguana*) are relevant because until now there are no studies showing POPs exposure background. In addition, because they are a basic part of rural communities' diet, meat intake may be a potential route of exposure to organic pollutants persistent for humans. Furthermore, because it is a tree species, it may be used to indirectly monitor air quality of some volatile organic compounds (VOCs) and semi-volatile ones as DDT and their metabolites, as well as some congeners of PCBs.
With crabs (*Uca* sp.) there are a few exposure studies to POPs. De Sousa et al., (2008) that registered POPs concentrations in crab eggs (*Chasmagnathus granulata*) in different Brasil stereos. Concentrations of DDE, DDT, γ-HCH, PCBs y total POPs are higher (35.95; 0.38; 3.52; 286.27 and 339.68 ng/g) than those reported in the present work. The previously said may be due to the fact that the matrix analyzed by de Sousa et al., contains a greater quantity of lipids, and crabs evaluated in this study (*Uca* sp.) were not in reproductive stage. Bayen et al., (2005) a study was conducted in a Singapure's mangrove swamp where a thropic web was established and the thunder crab (*Myomenippe hardwicki*) was one of the species presenting high concentrations of POPs. Falandysz et al., (2001) also used crabs (*Carcinus means*) to evaluate the exposure to organochlorine pesticides. It is complicated to make a comparison between crab species because they have different etiology and habitants, however, because most crab species are detritivores, we consider them as a good option to do studies related to POPs exposure.
There are several studies on earthworms with ecotoxicological background mainly focused on effects at population levels (lethality), at both laboratory controlled conditions (Heimbach, 1984; Ma & Bodt, 1993; Kula, 1995, Morrison et al., 2000) as in field ones (Thompson, 1970; Tomlin, 1981; Edwards & Brown, 1982; Haque & Ebing, 1983; Potter et al., 1994; Espinosa-Reyes et al., 2010), however, there are a few studies where exposure to POPs are evaluated, Jones and Hart (1998) revised works related to exposure of different earthworms species to various pesticides–Benomyl, Carbaryl, Carbendazim, Carbofuran, Chlordane, Methiocarb, Parathion, Pentachlorophenol, Phorate, Propoxur, Thiophanateme-. These same authors mentioned that earthworms of the *Eisenia* type are the most resistent to the pesticides previously mentioned. Morrison et al., (2000) evaluated the bioavailibility of DDT, DDE, DDD y Dieldrin in soil samples with different age of use of the mentioned pesticides. They exposed *Eisenia* earthworms. The concentrations registered by Morrison et al., (2000) are high when compared with the registered in this study (DDT 28.3±8.4 & DDE 3.77±0.48 mg/Kg tissue). Based on these backgrounds and results registered in this study, it is possible to postulate the Eisenia earthworms as POPs biomonitors in terrestrial ecosystems because earthworms play a major role in facilitating pivotal interactions within ecosystems through the mixing and translocation of soil constituents, or serving as a conduit for contaminants to predators at higher trophic levels (Harris et al., 2000; Langdon et al., 2003). Earthworms have been used extensively in ecotoxicology like biomonitors (Fitzpatrick et al., 1992; Goven et al., 1993; Reinecke & Reinecke, 1998; Espinosa-Reyes et al., 2010) to assess the effects of diffuse contaminants present in soils.
Finally, when doing a POPs biomonitoring in terrestrial ecosystems, it is important to take into account the following factors: a) species susceptibility difference; b) soil type; c) species behavior; d) exposure time; e) pollutant toxicity; f) when using pesticides consider the applying method.

3.2 Aquatic ecosystem

Because fish are organisms with a wide movement range, the sample was taken within an approximate area of 8 Km^2. Thirty-one fish from five species were caught: *Centropomus parallelus*, *Mugil cephalus*, *Eugerres axillaris*, *Oreochromis* sp, *Ariopsis felis* and thirty organisms of one crustacean species *Callinectes* sp., for quantification of POPs.

From de 29 quantified compounds in the simple, only were detected concentrations of HCB, α-, β-, γ-HCH, DDT, DDE, mirex and 6 congeners of PCBs (52, 101, 105, 118, 138, 153) (Table 2). Most concentrations registered were of β-HCH, α-HCH and mirex. HCB was only detected in 26% of the samples and just on species *Mugil cephalus y Callinectes* sp. El γ-HCH and DDT were registered in a 32% and 10% of the samples respectively. Pollutants α-HCH, β-HCH, DDE, mirex and PCBs were detected in a 100% of the samples. Species where a higher number of compounds were found are *Callinectes* sp, *Ariopsis felis* y *Eugerres axillaris* (8, 7 y 6 respectively), however, most concentrations were found in *Callinectes* sp and *Eugerres axillaris*. According to these results, POPs concentrations in muscular tissue by species decrease in this order β-HCH > α-HCH > mirex > DDE > Total PCBs > γ-HCH > DDT > HCB.

ECOSYSTEM	SPECIES	HCB	α-HCH	β-HCH	γ-HCH	DDT	DDE	Mirex	ΣPCBs	ΣPOPs
AQUATIC	Cepa	N.D.	1.2	0.1	N.D.	N.D.	0.1	0.3	0.2	1,8
	(n=9)		(0.05-5.3)	(0.05-0.5)			(0.05-0.5)	(0.05-0.6)	(0.05-0.4)	(0.5-5,9)
	Muce	0.1	0.2	0.3	N.D.	N.D.	0.2	0.3	0.1	1.0
	(n=7)	(0.05-0.2)	(0.05-0.3)	(0.05-0.7)			(0.05-0.5)	(0.2-0.4)	(0.05-0.2)	(0.6-1,5)
	Euax	N.D.	0.4	1.7	0.1	N.D.	0.1	0.6	0.1	2.9
	(n=7)		(0.03-1.9)	(0.05-3.8)	(0.05-0.3)		(0.05-0.1)	(0.1-0.9)	(0.1-0.2)	(0.9-4.7)
	Orsp	N.D.	0.3	N.D.	N.D.	N.D.	0.2	0.4	N.D.	0.8
	(n=5)		(0.2 -0.4)				(0.1-0.2)	(0.3-0.5)		(0.7-1.0)
	Arfe	N.D.	0.3	N.D.	0.1	0.1	0.1	0.5	0.3	1.3
	(n=3)		(0.2 -0.5)		(0.05-0.1)	(0.05-0.1)	(0.05-0.2)	(0.4-0.7)	(0.1-0.6)	(1.1-1.5)
	All fishes	0.1	0.6	0.9	0.1	0.06	0.2	0.4	0.2	1.7
	(n=31)	(0.05-0.2)	(0.03-5.3)	(0.05-3.8)	(0.05-0.3)	(0.05-0.1)	(0.05-0.5)	(0.05-0.9)	(0.05-0.6)	(0.5-5.9)
	Casp	0.2	1.2	1.6	0.2	0.1	0.4	1.3	0.3	14.8
	(n=4)*	(0.05-0.5)	(0.3 -2.4)	(0.05-5.2)	(0.05-0.2)	(0.05-0.1)	(0.1-0.9)	(0.9-1.7)	(0.1-0.7)	(13.3-17.6)

Values represent the mean and range. Cepa: *Centropomus parallelus*, Muce: *Mugil cephalus*, Euax: *Eugerres axillaris*, Orsp: *Oreochromis* sp, Arfe: *Ariopsis felis,* Casp: *Callinectes* sp. N.D.: non detected

Table 2. Concentrations of persistent organic pollutants in muscle tissue (ng/g wet weight) from aquatic wildlife collected in Coatzacoalcos, Veracruz.

Out of the *Ariopsis felis* fish species the highest concentrations of PCBs were registered and it is the only one that presented DDT, *Mugil cephalus* is the only species in which HCB was detected and it presented the highest concentrations of DDE, *Eugerres axillaris* showed the highest concentrations of lindane and α, β-HCH's, finally *Oreochromis* sp registered the lowest load of POPs in comparison to the other species.
Concentrations of β- HCH and α-HCH were the most abundant among the evaluated species, which matches with what was registered by Lee et al., (1997) and Yim et al., (2005) in other fish species. This can be explained because β-HCH and α-HCH have a greater bio-concentration factor (log BCF 2.8 and 2.5 respectively) in aquatic animals and they are more persistent than γ-HCH (log BCF 1.2) (Willett et al., 1998). Even though technical grade HCH has a greater constitution of α-HCH (60-70%), concentrations of β-HCH and α-HCH were very similar, this can be due to the fact that α-HCH has a high volatility (Henry' law constant 6.68 x 10-6 atm-3/mol) and environment degradation, which can cause low persistency (Yim et al., 2005). Another possible explanation is that HCH metabolized and excreted faster than β-HCH (Takazawa et al., 2005).
DDT was only detected in *Ariopsis Felis* while DDE was detected in all species. Most concentrations were registered in detritivores fish. As it was mentioned, DDT was widely used in Mexico for malaria control and because of its toxic effects, its use was eliminated. Chemical and biological processes transformed DDT into DDD and DDE, particularly DDE has been the most registered in biota (Takazawa et al., 2005). Registered concentrations in fish from the zone indicate that they are mainly exposed to residual DDT and to its degradation products. However, as it was seen, DDT/DDE relation in sediment suggests current use, reason why it is expected to have greater concentrations in the tissues, which suggests that fish from the zone have the capacity to eliminate DDT fastly.
Furthermore, quantification of polybrominated diphenyl ethers (PBDE) was done in 30 fish that belong to different species (*Aplodinotus* sp, *Centropomus parallelus*, *Mugil cephalus*, *Eucinostomus* sp, *Gobiomorus* sp, *Menticirrhus* sp and Torombolo –common name-), in these organisms, there were concentrations of 6 congeners registered (PBDE 47, PBDE 100, PBDE 99, PBDE 154, PBDE 153, PBDE 209) (Table 3). Considering all species, the order of concentration of congeners found was 47>41>154>209>153>100, being *Eucinostomus* sp species where the highest concentrations were found and in Torombolo species the lowest ones.
Within this context, aquatic ecosystems are highly vulnerable because of their tendency to accumulate concentrations relatively greater of pollutants coming from terrestrial ecosystems surrounding them, as well as those from direct entries (downloads), in that, regardless of its source of entry into the environment, aquatic systems are frequently deposits for a great variety of chemicals. Pollution in these environments can have negative effects over aquatic life (example: alteration of reproduction and decreased of species) as well as directly or indirectly affect human health and threat food safety. (Jha, 2008).

3.3 Wetland ecosystem

In Table 4 are presented the levels of persistent organic compounds detected in the different animal species from the Coatzacoalcos wetland. When monitoring them, 6 organochlorine pesticides out of the 14 ones analyzed were detected and 13 polychlorinated compounds biphenyls (PCBs) out of 21. All captured organisms from the different species presented detectable levels of at least 3 persistent organic compounds: DDE, Lindane y PCBs (data not shown, found in giant toads' adipose and hepatic tissue). Generally, the pattern of pollutants presence in giant toads' muscular tissue was ∑DDT> ∑HCH> Mirex > HCB; and for the turtles and crocodiles' case, the exposure levels of PCBs and DDE in serum were similar. It must be said that lindane and DDT concentrations in crocodiles were higher.

SPECIES	PBDE 47	PBDE 100	PBDE 99	PBDE 154	PBDE 153	PBDE 209	Σ PBDEs
Mesp (n=7)	3.2	1.9	4.5	0.3	0.7	0.7	11.3
	(0.5-6.5)	(0.5-4.3)	(1.0-8.0)	(0.04-0.5)	(0.3-1.2)	(0.3-1.2)	(3.8-19.7)
*Eu*sp (n=2)	12.03	3.7	2.6	18.0	10.2	13.2	59.7
	(7,7-16,4)	(0,8-6,5)	(1,7-3,5)	(9,5-26,5)	(1,5-9,2)	(8,6-17,7)	(43,1-76,2)
Muce (n=7)	5.5	1.6	5.4	1.3	1.1	0.9	15.8
	(0,2-24,8)	(0,1-2,9)	(0,5-10,6)	(0,03-4,2)	(0,1-2,0)	(0,1-2,2)	(1,0-45,1)
Gosp (n=1)	2.9	2.3	8.8	0.8	1.2	1.4	17.3
Apsp (n=3)	1.9	0.7	2.4	0.2	0.3	0.3	5.7
	(1,1-2,7)	(0,5-1,0)	(1,21-3,7)	(0,1-0,2)	(0,1-0,4)	(0,1-0,6)	(3,2-8,7)
Torombolo (n=2)	2.0	0.4	1.3	0.1	0.2	0.2	4.3
	(1,3-2,8)	(0,2-0,5)	(0,8-1,9)	(0,1-0,2)	(0,1-0,3)	(0,1-0,3)	(2,6-6,0)
Cepa (n=8)	2.2	1.0	3.7	0.2	0.5	0.5	8.1
	(1,0-4,0)	(0,5-1,4)	(0,8-5,5)	(0,1-0,5)	(0,2-0,6)	(0,1-0,8)	(3,1-12,6)
All fish (n=30)	4.2	1.7	4.1	3.0	2.0	2.5	17.5
	(0.2-24.8)	(0.1-6.5)	(0.5-10.6)	(0.03-26.5)	(0.1-9.2)	(0.1-17.7)	(1.0-76.2)

Values represent the mean and range. Mesp: *Menticirrhus sp.*, Eusp: *Eucinostomus sp*, Muce: *Mugil cephalus*, Gosp: *Gobiomorus s*, Apsp: *Aplodinotus sp*, Torombolo: -common name-, Cepa: *Centropomus parallelus*.

Table 3. Concentrations of polybrominated diphenyl ethers (ng/g lipid) in fish muscle tissue from species collected in Coatzacoalcos, Veracruz.

ECOSYSTEM	SPECIES	HCB	α-HCH	β-HCH	γ-HCH	DDT	DDE	Mirex	ΣPCBs	ΣPOPs
WETLAND	Rhma	0.08	0.75	0.31	0.26	ND	1.51	0.39	ND	3.1
	(n=12)	(N.D.-0.15)	(0.41-2.05)	(N.D.-1.24)	(N.D.-0.53)		(0.05-7.8)	(N.D. 0.67)		(0.84-9.56)
	Crmo‡	N.D.	N.D.	N.D.	64.5	59.2	11.5	N.D.	2.7	76.0
	(n=2)				(N.D. - 64.5)	(N.D. - 59.2)	(N.D. - 11.5)		(2.5 - 2.7)	(72.7 - 79.2)
	Trsc‡	N.D.	N.D.	N.D.	3.5	N.D.	8.8	N.D.	3.0	7.0
	(n=4)				(N.D. - 4.58)		(N.D. - 8.78)		(N.D. - 5.83)	N.D. - 14.61

Values represent the mean and range. Rhma: *Rhinella marina*, Crmo: *Crocodylus moreletii*, Trsc: *Trachemys scripta*. * Pool samples, ‡Blood samples, N.D.: non detected

Table 4. Concentrations of persistent organic pollutants in muscle tissue (ng/g wet weight) and blood serum (ng/ml) from wildlife collected in Coatzacoalcos, Veracruz.

The DDT/DDE relation obtained in toads' tissue was lower than 1.0 which suggests the organism's ability to metabolize the parental compound to DDE. Studies on amphibians, especially the toads' family (Bufonidae), has proven these organisms capacity to accumulate high concentrations of DDT and its metabolites. It should be said that these studies have also shown that proportions of DDE and DDT were higher than those of the parental compound. In our case, DDD concentrations in tissues were not quantified, so exposure of toads from the Coatzacoalcos region to DDT and its metabolites may be underestimated. Registered DDE concentrations in this research are comparable with those found in adult anuran coming from other polluted sites (Table 4 and 5). There are records of ∑DDT concentrations and their metabolites (mainly DDE) up to 3480 µg/g of fat in samples of *R. clamitans* coming from the wetlands from the south of Ontario (Harris et al 1998); in our study, we found concentrations that go up to 3094.5 µg/g of fat in samples of giant toad's liver (data not shown). POPs exposure works in fresh water turtles' populations are scarce; studies done in Terrapin (*Malaclemys terrapin*), Snapping turtle (*Macrochelys temminckii*) and Common musk (*Sernotherus odoratus*) have determined levels of DDE of 0.73-21.7 ng/g wet mass (Basile, 2010, Moss et al., 2009, De Solla et al,. 1998). Several studies on marine turtles have proven levels of DDE of 0.06- 0.73 ng/g wet mass (Stewart et al 2011). Our study shows comparable levels between species of terrestrial and fresh water turtles. Crocodiles present the highest levels of DDT and DDE in plasma; be noted that higher organochlorine pesticides concentrations in the world (mainly DDE) have been found in the Crocodilia species due to their position in the trophic chain. Guillette et al., (1999) Reported DDE levels of 0.9- 17.9 ng/ml and DDT levels of 0.45 to 0.70 in plasma of alligator's residents at Apopka Lake in florida; our study shows comparable levels; even greater to the ones found in Apopka Lake.

The distribution general pattern of Hexaclorociclohexanos (HCH´s) observed in toads' tissues was α-HCH>β-HCH>γ-HCH and for the crocodiles and turtles' case, levels of γ-HCH were detected. In accordance with literature, general distribution patterns of HCH's in mammals, birds and fish are β-HCH>α-HCH>γ-HCH; this distribution pattern is mainly determined by the compound persistency, the exposure path, the species metabolism, and the trophic position (Willet et al., 1998). Our data contrast with the distribution pattern observed in other studies of environmental and biological matrix. Some possible explanations in order to interpret the presence of a higher proportion of γ-HCH is the chronic exposure to isomer of different routes, which can be noted with the found values in crocodile and turtle's plasma where isomer γ-HCH is the only detectable one (Table 4). El γ-HCH is more volatile in comparison with the other isomers, which implies an important transport by air, it is also the isomer with most solubility in water (Walker et al 1999). Coupled to the previously said, it is possible that other important exposure routes exist towards HCH's which have not been explored yet and which may significantly contribute to corporal load and its distribution to these animals. Even though differences in isomers proportions may indicate different sources, routes and times of exposure, for many species it is not clearly understood the influence of processes such as intake, distribution, metabolism and storage in the differences of isomer distribution in tissues (Willet et al., 1998). HCH's concentrations detected in this study are lower than those observed in other studies done on wild amphibians living in agricultural sites (Table 4 and 5). In the case of turtles and crocodiles, there was no useful information found to compare data with.

SPECIE	COMPOUND	CONCENTRATION	TISSUE	REFERENCE
Pseudacris crucifer	DDE	1001	Whole	Russell et al 1995
	DDT	160.6		
	α- HCH	0.37		
	β- HCH	1.37		
	γ- HCH	<DL		
Rana clamitans	DDE	0.58-45.0	Whole	Russell et al 1997
	HCB	0.08-0.49		
	∑DDT	1.24		Guilliland 2001
	∑HCH	0.12		
Rana perezi	DDE	<DL-190	Muscle	Rico et al 1987
	∑DDT	50-550		
	γ- HCH	<DL-10		
	∑DDT	35.4	Whole	Pastor et al 2004
	HCB	2.7		
	α, γ- HCH	0.5		
Rana pretiosa	DDE	91-173	Whole	Kirk 1988
	DDT	563-1750		
Rana mucosa	DDE	17-100	Whole	Fellers et al 2004
	α- HCH	<DL-4.9		
	γ- HCH	<DL-0.7		
Necurus maculosus	DDE	0.3-90.0	Whole	Bonin et al 1995
	DDT	<DL-8.3		
	∑HCH	<DL-10.1		
Necturus lewisi	DDE	60	Whole	Hall et al 1985
Chaunus arenarum	DDE	ND-4.5	Whole	Jofre et al 2008
	α- HCH	ND-5.6		
	β- HCH	ND-2.3		
	γ- HCH	ND-2.7		
Hypsiboas cordobae	DDE	1.1-1.7	Whole	
	α- HCH	3.9-5.4		
	β- HCH	3.0-7.3		
	γ- HCH	4.9-7.2		
Leptodactylus mystacinus	DDE	ND-6.0	Whole	
	α- HCH	3.5-6.9		
	β- HCH	0-8.9		
	γ- HCH	ND-5.7		
Melanophryniscus stelzneri	DDE	10.6	Whole	
	α- HCH	6.99		
	β- HCH	ND		
	γ- HCH	ND		
Odontophrynus occidentalis	DDE	0.8-1.8	Whole	
	α- HCH	1.3-4.1		
	β- HCH	1.0-2.7		
	γ- HCH	1.5-3		
Pleurodema tucumanum	α- HCH	4.7	Whole	
	β- HCH	4.1		
	γ- HCH	4.4		

DL-Detection limit, ND-Not detected

Table 5. Persistent Organic Pollutants concentrations (mg/Kg wet weight) measured in various amphibian species from different studies.

Mirex and HCB were pollutants found in less proportion in giant toads' tissues. There are a few studies related to Mirex and HCB exposure on adult amphibians. Russell y collaborators (2002) reported average concentrations of 0.26-1 ng/g tissue of HCB in cricket frogs (*Acris crepitans*) coming from 5 agricultural locations in Ohio USA; in table (Table 5) other studies are presented with levels of HCB which are higher when compared to the ones obtained in this study (0.14-0.67 ng/g of tissue). Detectable levels of Mirex and HCB in turtles and crocodiles were not found.

PCBs congeners detected in blood and tissue sample were 52,101, 105, 118, 138, 153, 156, 170 and 180, which corresponds with the reported in other studies as the most common for human and biological samples (Table 4). The PCBs congeners presence pattern observed in the giant toad's tissue is consistent with other studies on amphibians from other regions around the world (Loveridge et al., 2007, Russell et al., 1997); as well as in the found concentrations (Table 6). Studies conducted in diverse species of fresh water and terrestrial turtles have reported levels of PCBs totals of 5-414.8 ng/g wet mass (Basile, 2010, Moss et al., 2009, De Solla et al,. 1998) and for alligator populations there have been found levels of 1.54 ± 0.12 ng/ml (Guillette el al 1999); these levels are comparable with the ones obtained in this study. Detected congeners (except 52) correspond in greater proportion (>30%) to aroclor 1254, one of the most sold commercial mixtures in the world, which suggests that the origin of these compounds probably is related to the use of these oils in the region's industrial areas. Detected congeners are characterized for being some of the most persistent ones in the environment and for being absorbed in greater proportion in the organisms.

SPECIE	TISSUE	CONCENTRATION	REFERENCE
Rana clamitans	Carcass	2.8	Loveridge et al 2007
Various anurans	Carcass	151-4470	DeGarady y Halbrook 2003
Rana clamitans	-	7.51	Rusell et al 1997
Necturus maculosus	Carcass	113-1082	Bonin et al 1995
Rana pipiens and *Rana clamitans*	Carcass	50-112	Phaneuf et al 1995
Rana perezi	Muscle	50-1080	Rico et al 1987

Table 6. Total PCB concentrations (ng/g wet weight) measured in various amphibian species from different studies.

Amphibian and reptiles populations are declining at an alarming way in the world (Alford, 2020; Todd et al., 2010); some of the determining concomitant causes in this phenomenon is the exposure to toxic agents; let us note that reptile and amphibian toxicological information is growing, however, it is yet limited in comparison with other vertebrate groups (Sparling et al., 2010). Amphibians and reptiles may be exposed to a wide spectrum of toxic substances; pollutant accumulation in these organisms may be influenced by many factors (physiological, trophic, behavioral, etc.) at the same time, exposure may occur by different routes and in different environments during their life-time. It is known that these organisms can accumulate significant pollutant loads in their tissues, mainly of heavy metals and organic compounds. The caused effects by exposure to POPs of greater concern in reptile and amphibian populations are the endocrine disruption, DNA damage, and development abnormalities; some of the studies of greater impact over these effects have been found in these organisms. Ecological importance to maintain viable reptile and amphibian populations is determinant because these organisms are the link between terrestrial and aquatic ecosystems; at the same time they are mainly placed between intermediate links of trophic chains; they have also diversified and occupied a wide spectrum of ecological niches in different types of ecosystems; the presence of highly persistent, bioaccumulative and biomagnifying pollutants is a potentially dangerous situation worth to be evaluated in the Coatzacoalcos Veracruz ecosystems.

3.4 Trophic levels and bioaccumulation of POPs

In Figure 2, the integration of exposure to POPs in tissue (ng/g lip) of the evaluated species in each one of the ecosystems present in Coatzacoalcos; a trophic hypothetical web was established in the region. Results were classified according to the trophic level that species belong to. It can be observed that herbivores are the ones presenting the lowest POPs concentrations followed by carnivorous, omnivorous, and finally the ones with the highest concentrations are the omnivorous. This is explained because detritivores organisms are found in grater contact with contaminated matrix (as soil and sediment), while omnivorous organisms include a greater number of exposure routes (environment and food) and are, therefore, the ones presenting greater POP's concentrations in their tissues.

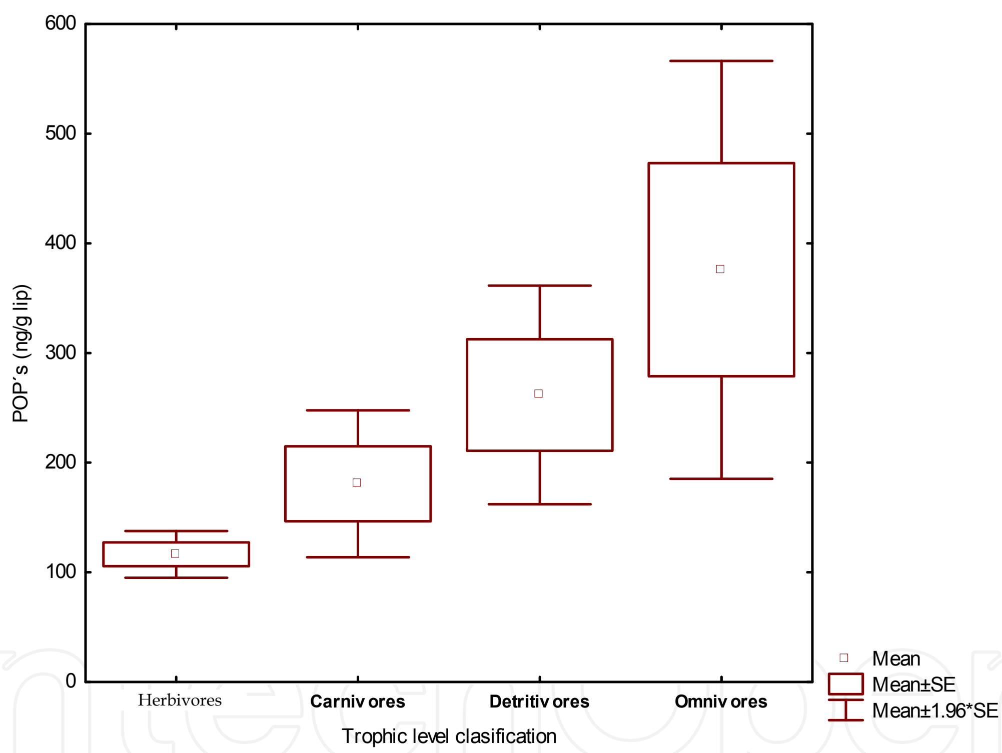

Fig. 2. Concentration Levels of POPs* in tissue taking into account the feeding habits of some species collected at Coatzacoalcos, Ver. *Corresponds to the sum of chloride compounds. Herbivores (iguana); Carnivores (Fish–Cep-); Detritivores (Fish –Arfe, Muce-Crab –Ucsp- and–Casp-); Omnivores (Fish –Tilapia-, Amphibian–Rhma-)

4. Conclusion

With the noted exception of HCB which was not found in the terrestrial ecosystem, traces of all other POPs were identified in species from all three ecosystems. Performing an analysis of feeding habits, it was learned that concentrations of POPs rise as follows: herbivores < carnivores < detritivores < omnivores. With these results it may be established that the region's biota is in fact exposed to diverse Persistent Organic Pollutants. In similar studies it has been found that exposure to POPs may cause several effects (Espinosa-Reyes et al., 2010; González-Mille et al., 2010). In addition, one must consider that Coatzacoalcos organisms are exposed to other types of pollutants in addition to POPs whose collective action could well increase the magnitude of the effects. On the other hand, as far as brominated compounds go, this is the first ever study on fish in the region. The described scenario leads us to ask ourselves what exactly is the risk to humans and wildlife in the region. The Outlook is not promising if you consider that these organisms are of paramount importance in the food chain both to humans and other species of wildlife.

Available data on concentrations of chemical substances in the environment and human beings as well as over effects of exposure to chemicals complex combinations is still scarce. Chemicals generally pose a risk for the environment; they are a rapidly growing pollution load including chemical compounds increasingly complex from which potential effects on public health and environment are probably known. It is estimated that between 70,000 and 100,000 chemicals are available in the market and this number is growing fast. Around 5,000 of these substances are being produced in high volumes, over one million tons a year. The biggest chemical producers are countries members of the OECD, but countries such as India, China, Brazil, South Africa and Indonesia are rapidly increasing their productions. One of the main reasons for the development and adoption of the REACH Regulation is that a great number of substances have been produced and marketed in Europe during many years, at times in very large quantities yet there is not enough information over the hazards for human health and the environment. The European Commission has estimated that in order to fill in information gaps related to toxic effects of that large number of substances it may be required the use of 9 million of lab animals with an approximate cost of 1.3 billion € to perform the necessary tests. A later estimate suggested that required tests would imply 54 million vertebrate animals and costs would grow up to 9.5 billion €. Within this context, wildlife bio-monitoring may be used to detect chemical pollution and evaluate the health of ecosystems, with species as systematic test models during the evaluation of risks associated to actual exposure routes. Wildlife species residing in polluted zones are exposed to pollutant complex mixtures through multiple paths that could hardly be evaluated in laboratory tests.

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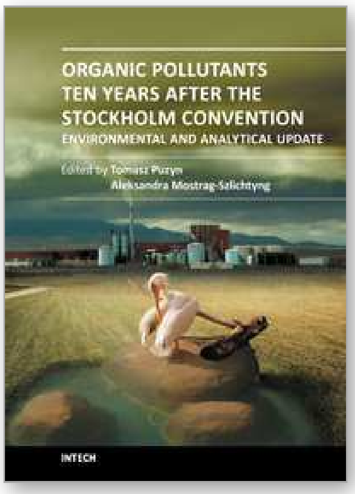

Organic Pollutants Ten Years After the Stockholm Convention - Environmental and Analytical Update
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Ten years after coming into force of the Stockholm Convention on Persistent Organic Pollutants (POPs), a wide range of organic chemicals (industrial formulations, plant protection products, pharmaceuticals and personal care products, etc.) still poses the highest priority environmental hazard. The broadening of knowledge of organic pollutants (OPs) environmental fate and effects, as well as the decontamination techniques, is accompanied by an increase in significance of certain pollution sources (e.g. sewage sludge and dredged sediments application, textile industry), associated with a potential generation of new dangers for humans and natural ecosystems. The present book addresses these aspects, especially in the light of Organic Pollutants risk assessment as well as the practical application of novel analytical methods and techniques for removing OPs from the environment. Providing analytical and environmental update, this contribution can be particularly valuable for engineers and environmental scientists.

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