IUCN-SSC Crocodile Specialist Group

Determination of polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCs) in swamp crocodiles (*Crocodylus moreletii*) and their prey in a lagoon system in the municipality of Emiliano Zapata, Tabasco, Mexico.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are compounds generated mainly by combustion processes, burning and fires and, in the case of organochlorine pesticides (OCs), they were intensively and extensively used in agriculture and livestock for pest control (Dupree and Ahrens, 2007; Albert and Viveros, 2018). Due to their persistence and high volatility these compounds can be found in almost all aquatic systems, generating toxic effects on organisms through the processes of bioaccumulation and biomagnification along trophic chains, mainly at the level of top predators such as the swamp crocodile (Crocodylus moreletii) (Ponce-Vélez, 2012). The state of Tabasco, Mexico, has extensive lagoons and river flows that provide high primary productivity, including industry (port and petrochemical), agriculture (fishing and aquaculture) and livestock. These activities have developed rapidly, presenting potential environmental challenges and threats to aquatic ecosystems (Pinkus-Rendón and Contreras-Sánchez, 2012).

The objectives of this research were 1) to analyze C. moreletii caudal scales and prey tissue for organic contaminants, 2) to evaluate the relationship of consumers to bioaccumulation and trophic transfer of compounds at each trophic level sampled, 3) to evaluate the influence of the lipids of the species studied with respect to the trophic distribution of PAHs and OCs.

2. Methods

2.1. Sample collection

A total of 55 organisms of 11 species were captured in the town of Nuevo Pochote belonging to the municipality of Emiliano Zapata, Tabasco. We considered (1) the conservation status of the species according to IUCN (2018), NOM-059-SEMARNAT-2010 (DOF, 2010) and CITES, (2021). Leaves of aquatic plants (*Oryza sativa, Salvinia auriculata*) and herbivorous mollusks (*Pomacea flagellata*) were collected directly. Detritivorous (*Vieja bifasciata*), omnivorous (*Astyanax finitimus*), zoobenthivorous (*Thorichthys pasionis, Ictalurus meridionalis*), and piscivorous fish (*Atractosteus tropicus*) were captured using trawls and traditional techniques with the help of local fishermen. Omnivorous amphibians (*Lithobates brownorum*) were collected using nets on night transects. Omnivorous turtles (*Trachemys venusta*) were captured directly, and crocodiles (*Crocodylus moreletii*) were captured using a snare attached with a pole. A sample of interdigital membrane was obtained from turtles and caudal scales from crocodiles, and the organisms were immediately released.

Liver and hepatopancreas were sampled in fish and mollusks by dissection. Samples were frozen (-20°C) during transport to the Persistent Organic Pollutants (POPs) laboratory at the EPOMEX Institute located in the state of Campeche. At the laboratory, the samples were dried in an oven (45°C/24 h) to a constant weight, and the dry weight (d.w.) of the total samples was recorded.

2.2.PAHs and OCs analysis

Leaf extraction was performed by the method of Wang et al. (2007) by microwave digestion (MarsXpress, 6 CEM). In biological samples the extraction was performed following the method of Zhao et al. (2005) by sonication in an ultrasonic equipment (Fisher Scientific, FS60). The concentrated extracts were passed through a chromatographic column with silica gel, alumina, sodium sulfate anhydride and solvents in sequence: acetone, hexane, and dichloromethane HPLC (30 mL), bringing the product to a final volume of 1 mL. Analysis of PAHs and OCs was performed using the Thermo Scientific TRACE 1300 (GC-MS/MS) coupled to a TSQ 8000 triple quadrupole MS and a TriPlusTM RSH automatic liquid sampler (ThermoFisher Scientific, Austin, TX).

For the interpretation of the PAH source, the number of rings was considered, where a predominance of 2- to 3-ring PAHs indicates a petrogenic origin and 4- to 6-ring PAHs of pyrogenic origin (Neff et al., 2005; Ortiz-Salinas et al., 2012). The OCs were grouped according to their chemical family: ∑HCHs (α+ β + γ + γ + δ HCH), ∑dienes (aldrin + dieldrin + endrin aldehyde + endrin ketone), ∑endosulfans (endosulfan I + II + endosulfan sulfate), ∑DDDTs (p,p DDE + p,p DDD + p,p DDT), ∑heptachlor (heptachlor + heptachlor epoxide) and methoxychlor.

2.3. Lipid content

Para la normalización de lípidos se utilizó un extracto de la submuestra biológica para la determinación gravimétrica. Para cada muestra seca se adicionó un volumen conocido de hexano (1 mL) y se pesaron 3 gotas en una balanza analítica.

2.4. DATA ANALYSIS

Statistical analyses were performed using STATISTICA software (StatSoft Inc., 2005). The statistical significance level was defined as $p \leq 0.05$. To know if there are significant differences between PAHs and OCs concentrations with respect to trophic level, a Kruskal-Wallis analysis was performed. To find out if the percentage of lipids has an influence on this trophic transfer, a Spearman correlation analysis was carried out.

3. Results and discussion

3.1.PAHs and OCs by Trophic Level (TL)

The results indicated that the OCs with the highest presence in C. moreletii were: ∑dienes (56 ng/g) > ∑DDTs (7.7 ng/g) > ∑endosulfans (6 ng/g) > methoxychlor (5.9 ng/g) > ∑HCHs (3.5 ng/g) > heptachlor (3.2 ng/g). While 2- to 5-ring PAHs were the most prevalent at both sites: acenaphthene (1,135 ng/g), fluorene (270 ng/g), naphthalene (152 ng/g) and benzo[a]pyrene (109 ng/g). The results indicated a bioaccumulation process of organic compounds in the swamp crocodile.

The highest concentrations of ΣHAPs occurred mainly in zoobenthivorous, omnivorous and carnivorous species, with *T. pasionis, T. venusta* and *C. moreletii* species representing each group (Table 1). The trophic levels studied showed a predominance of petrogenic ΣHAPs (2 and 3 rings) and in smaller proportion pyrogenic (4 to 6 rings), Figure 1. The

concentrations of ΣHAPs did not show significant difference between trophic levels ($p > 0.05$). A linear relationship was indicated for ΣHAPs values with respect to trophic levels ($r = 0.072$; $p < 0.05$).

With respect to OCs, the average concentrations at the trophic levels evaluated showed the following order of predominance of OCs: methoxychlor (923 ng/g) > ΣHCHs (399 ng/g) > Σdienes (548 ng/g) > ΣDDTs (523 ng/g) > Σendosulfans (170 ng/g) > Σendosulfans (170 ng/g) > Σheptachlor (53 ng/g). (Figure 1). Biomagnification at the upper levels is suggested from the feeding type of the organisms. Zoobenthivorous species usually feed on benthic invertebrates that are in greater contact with the contaminated detritus, omnivorous species in addition to the detritus include certain types of plants to their diet leading to greater routes of exposure (Ponce-Vélez, 2012). Crocodiles are opportunistic predators with carnivorous habits located at the highest levels of food chains, therefore, the presence of contaminants in their tissues may be the result of trophic transfer through their prey, generating a process of bioaccumulation in their body reserves (Arukwe et al., 2015).

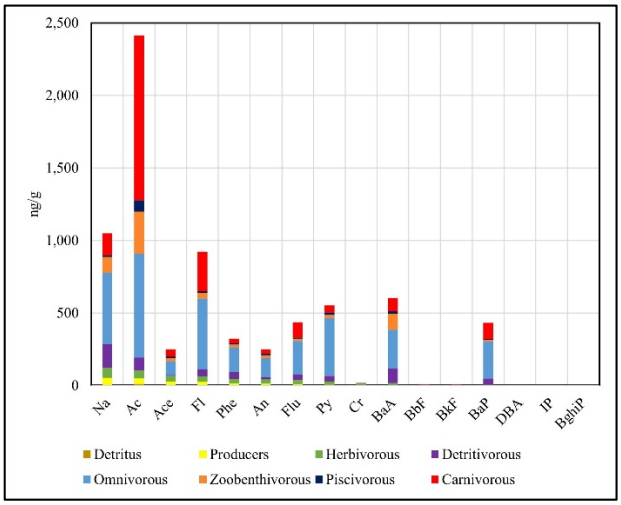


Fig. 1. Concentrations and predominance of PAHs (ng/g d.w.), categorized according to trophic level in Nuevo Pochote, Emiliano Zapata, Tabasco.

Table 1

Concentrations of ΣPAHs and OCs (ng/g d.w.), mean (±SD) in aquatic species from Nuevo Pochote, Emiliano Zapata, Tabasco, categorized according to Trophic Level (TL).

Specie	Trophic level	ΣPAHs	ΣHCHs	Σendosulfans	ΣDDTs	Σdienes	Σheptachlor	methoxychlor
Sediment	Detritus	3.5±1.6	0.6±0.4	0.07±0.02	0.6±0.4	0.5±0.2	0.04±0.04	1.7 ±0.8
Oryza sativa *Salvina auriculata*	Producers	10.3±2.3	0.03±0.01	0.03±0.01	0.7±0.5	0.26±0.13	0.104±0.01	0.09±0.07
Pomacea flagellata	Herbivorous	13.8±8.3	1.2±2.2	0.1±0.09	2.0±1.1	1.6±0.3	0.1±0.08	0.6±0.7
Vieja bifasciata	Detritivorous	118.7±162	11±8.3	2.3±1.2	13.4±13.3	13.6±7.1	1.7±1.2	27.0±22.7
Lithobates brownorum *Trachemys venusta* *Astyanax finitimus*	Omnivorous	648.7±199	40.1±19	12±13.2	209±229	113.3±41	5.8±3.7	101±191.2
Thorichthys pasionis *Ictalurus meridionalis*	Zoobenthivorous	1148±1772	342±391	149±241.6	287±323	362±516	42.3±56.5	786±1041
Atractosteus tropicus	Piscivorous	34.8±51.7	0.6±0.8	0.1±0.1	2.6±2.9	0.9±1.2	0.01±0.01	0.6±0.8
Crocodylus moreletii	Carnivorous	405± 667	3.5±4.4	6±11	7.7±12	56±97.5	3.2±5.5	6±8.8
Total		2382±2863	399± 426	170± 267	523±582	548±664	53.2±67	923±1266

3.2.Lipid content

According to the results, the species evaluated with the highest percentage of lipids were *T. pasionis* and *C. moreletii*. Non-significant correlations were found between lipid percentage and ΣHAPs concentration in the trophic groups. However, if there was a significant correlation ($p < 0.05$) between species and OCs concentrations (Figure 2). The organisms evaluated contained percent more lipids 50%, inferring that OCs concentrations bioaccumulate according to the size of the organisms and the lipid content in them, which in turn suggests a trophic transfer from the feeding type. Wang and Wang (2005) reported that DDT uptake in aquatic organisms of different trophic levels (phytoplankton, copepods, fish, reptiles) according to diet type was 10 to 29% in copepods and 72 to 99% in fish and reptiles.

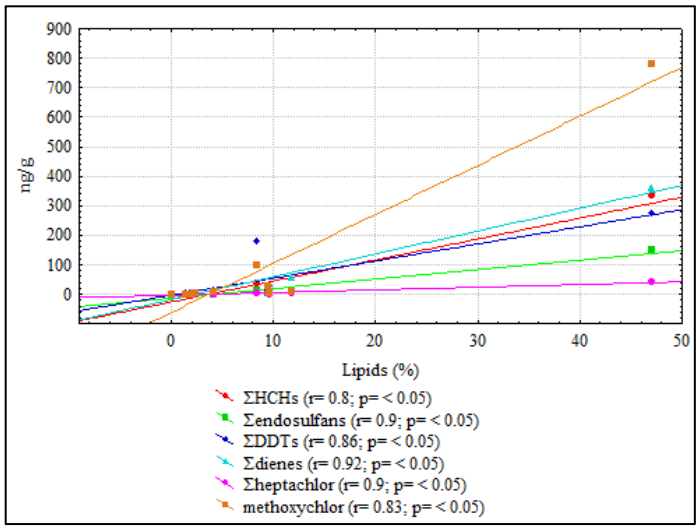


Fig. 2. Relation of OCs concentrations (ng/g d.w.) and percentage of lipids (%) present in species by Trophic Level (TL), from Nuevo Pochote, Emiliano Zapata, Tabasco.

Conclusions

The presence of PAHs of petrogenic and pyrogenic origin was confirmed, as well as the presence of four groups of dominant OCs: methoxychlor, ΣDDDTs, ΣHCHs and Σdrins in C. moreletii and aquatic organisms evaluated in the municipality of Emiliano Zapata, Tabasco. A process of bioaccumulation and trophic transfer as trophic levels ascend is suggested by the significant relationship between feeding type and lipid content of the evaluated species. The zoobenthivore, omnivore and carnivore groups had the highest amounts of contaminants, as they have a varied diet and thus greater routes of exposure. The presence of ΣHAPs may be influenced by oil activities, forestry, RTQ practices, while the presence of OCs is derived from agricultural and public health activities in the region despite their restriction.

Acknowledgments

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