Genotoxicity by Pahs In Shrimp (*Litopenaeus Vannamei*) and Its Impact on The Aquaculture of Two Coastal Ecosystems of The Gulf of California, Mexico

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***Abstract* — During the last decades, aquaculture of several species have growth vertiginously around the world. In Mexico the shrimp aquaculture has been the most important. About 73-75 % of shrimp hatcheries are in coastal ecosystems of the states of Sonora and Sinaloa, located along the Gulf of California. In this States there is not oil industry; however, several industries and other activities discharge petroleum derivatives (imprudently or accidentally) into coastal waters; as happens in Teacapan estuary and Huizache-Caimanero lagoon. The aim of this work was to quantify the levels of PAHs in water of these ecosystems, and to evaluate the genotoxic damage to shrimp, under laboratory conditions. Water samples were taken during rainy and dry months from both coastal systems, and then analyzed by Gas Chromatography (GC). Once known the PAHs concentrations, lots of seven juvenile shrimp were exposed to sub-lethal concentrations of Naphthalene, Phenanthrene, Chrysene, Fluorene, Anthracene, Pyrene, Fluoranthene, Benzo(b)fluoranthene and Benzo(a)pyrene during 21 days, since these were the most frequently PAHs found. At end of exposure period, genotoxicity was evaluated by Comet assay, and presence of micro-nucleus in shrimp haemocytes. Results demonstrated genotoxic damage by presence of comets, and micro-nucleus more frequently in exposed shrimps than controls. Also, a growth decrease was observed in exposed shrimps. These results, indicate potential risk for shrimp aquaculture in Sinaloa and human health, since shrimp is exported and consumed locally, and because in some cases, experimental PAHs concentrations were lower than concentrations of some PAHs found in water of Teacapan estuary and Huizache–Caimanero lagoon.**

***Index Terms* — aquaculture, genotoxicity, PAHs pollution, shrimp.**

I. Introduction

In Mexico as in other countries, the aquaculture activities have grown vertiginously during last decades. In 2018, the worldwide aquaculture production was estimated in 114.5 million tons, with a value of 263.6 billion of US dollars [1]. The cultivated shrimp in ponds under controlled conditions, is one of more important aquaculture species, with an estimate world production of 4.7 million tons in 2018 [2]. Although Mexico registered a severe decrease production in 2013, a growth of 180,000 tons is expected for 2021[2]. The same source reported that Mexico holds the sixth place in the world as shrimp producer after China, Vietnam, Ecuador, India and Indonesia. In Mexico, 73-75 % of shrimp hatcheries are located along coastal ecosystems of Sonora and Sinaloa States [3], which are located into the Gulf of California Fig.1. As currency income, the shrimp aquaculture is the most important in the country; this yielded approximately 778 million of US dollars, from which 56.3 % was produced in Sinaloa, during 2017 [3]. The same source report that for each produced ton of shrimp, 671 Kg were produced in hatcheries, and 329 Kg fished in estuaries, coastal lagoons and adjacent sea.

Fig. 1. In Mexico, 73-75% of shrimp hatcheries are on the coast of Sinaloa and Sonora States, located along the Gulf of California. Three sampling sites were selected in the studied coastal systems (Huizache-Caimanero lagoon and Teacapan estuary) based on accessibility to reach the sites, and shrimp aquaculture and fishing activity.

On the other hand, although in the Gulf of California there are not oil industries, since all them are located in the Gulf of Mexico, previous studies have reported presence of Polycyclic Aromatic Hydrocarbons (PAHs) in coastal systems in the Sinaloa State [4], [5]; therefore, the presence of PAHs in this ecosystems becomes a problem for shrimp aquaculture, since all the hatcheries are located along the coastal zone, and take water to fill their shrimp ponds from these ecosystems; consequently, this becomes a risk for shrimp and human health. Therefore, the objective of this study was to evaluate the concentrations of PAHs in the waters of Teacapan estuary and Huizache-Caimanero lagoon; and then, to evaluate the genotoxic effect of these compounds in shrimps; because in last years, severe losses in the shrimp hatcheries, have been reported; probably due to intense traffic of fishing boats and other activities, such as shrimp freezing factories, agriculture equipment, shrimp hatcheries pumps and some industries, which use diesel,

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gasoline, and other petroleum products as energy source, and also by diverse chemicals, who wastes could be discharged to coastal ecosystems. Other authors have reported similar pollution problems in coastal systems [6], [7].

The PAHs are a group of compounds made up of several benzene and other aromatic rings linked together. They are produced by the incomplete combustion of fossil fuels (gasoline, diesel, fuel oil, coal, etc.), and other processes such as the burning of garbage, agricultural waste, etc. [8]. They could be also generated during natural processes such as forest fires and volcanic activity. The PAHs are widely distributed in almost all ecosystems of the planet. In the marine environment, the PAHs are found mainly in coastal areas [9], [5]. The entrances via of these pollutants to coastal ecosystems are diverse, such as continental runoff, atmospheric deposition, municipal and industrial effluents, and often by direct discharges [10], [11], [12]. The PAHs can enter to aquatic organisms by ingestion, respiration, filtration and dermal absorption; and due to their slow degradation process in the organisms, PAHs are commonly accumulated in tissues and muscles of aquatic organisms [13]. The acute toxicity in mice to light PAHs is moderate, ranged from 75 to 150 for Pyrene and Fluoranthene, while heavier PAHs have a higher toxicity; around 3 to 10 (mg/kg/day) for Benzo(a)pyrene and benzo(b)fluoranthene [14], [15]. The most critical effect of PAHs in mammals is due to their carcinogenic and genotoxic potential; for example, the Benzo (a) anthracene, Benzo (a) pyrene and Dibenzo (a, h) anthracene are considered potentially carcinogenic in humans [16], [17]. There are some works that assess the introduction routes of PAHs to coastal environments [18] and about the environmental degradation and its speciation process [19], [20]. However, the literature on PAHs contamination in the coasts of the Gulf of California, is scarce, and there has been little attention to toxicity of these pollutants on aquatic organisms.

II. Material And Methods

In order to know the water concentrations of PAHs, water samples were collected in three places of both ecosystems, during January, March, July and September, which correspond to the dry and rainy seasons respectively. The samples were collected using 4 L glass bottles. Previously, the bottles were rigorously washed with soap and water, and then, rinsed with distilled water and acetone. The collected samples were transported in coolers to laboratory where they were kept at 2-3 °C until processing. The PAHs in water were extracted using a liquid-liquid system. The extraction system consists of a balloon flask where n-hexane is vapored by a heat mantle; the n-hexane in vapor phase passes through the water sample, by a small bubbler located in bottom of extractor vessel. After, the n-hexane in the upper layer of the extractor with PAHs dissolved, drop by a glass tube arm connected to upper part of extractor and to balloon flask; the PAHs dissolved in n-hexane are adsorbed by an activated carbon trap, packed in a segment of descendent glass tube. The n-hexane without PAHs is re-evaporated in the balloon flask and passed back through the water sample. The n-hexane recycling process was carried out during 4 h. The PAHs absorbed by activated carbon were re-dissolved with fresh n-hexane. The extracts were clean-up passing them through packed columns (3,3,3,4) g of Silica-gel, Alumina, Florisil and anhydrous Na_2SO_4; then, extracts were concentrated until 2 ml, using a Rota vapor Buchi® R-210. The concentrates were carried out to dryness by a N_2 gentle flow, and then re-dissolved in fresh n-hexane. The PAHs analysis of clean extracts, were carried out by Gas Chromatography (GC), using an Agilent 6890® chromatograph (Palo Alto, CA), in splitless mode, fitted with a Flame Ionization Detector (FID), and a WCOT capillary column DB-5 (30 m x 0.25 mm OD) 5% phenyl methyl silicone. The operating conditions were as follows: an initial temperature of 60 °C during 3 min, a ramp of 8 °C/min until 320 °C keeping this temperature by 4 min. The Injection and detector temperature were 250 °C and 330 °C respectively. Nitrogen (purity ≥99.7%) was used as carrier gas at a constant flow rate of 2.5 ml/min, and constant pressure (15 psi) during all run. Hydrogen was used as fuel gas with a flow rate of 40 ml/min and dry air as oxidant gas, with a flow rate of 450 ml/min. The identification of PAHs congener was carried out comparing the retention time (RT) peaks in the samples chromatograms vs. the RT peaks of reference standard chromatogram of PAHs; corresponding to 16 parent PAHs priority [21]. The congener quantification was performed by the peak/area normalization method. The identification and quantification procedure was carrying out by Agilent ChemStation® software, installed in a computer attached to chromatograph. The reference standard was purchased in Sigma-Aldrich de Mexico, and other items were supplied by Agilent Technologies of Mexico (Mexico City).

During last years, several methods have been utilized to evaluate genotoxic damage in diverse aquatic species; however, the comet assay method for detecting DNA strand breaks, and the micronucleus count, as an index of chromosomal damage are the most used and validated methods. Groups of seven healthy juveniles shrimp (6.5-7.5 g each) were distributed in 20 L aquariums. The salinity was adjusted to 26-27 PSU (Practical Salinity Unit), adding filtered fresh water to filtered seawater. The water temperature of aquariums oscillates from 27-28 °C, during experimentation time. Constant aeration was supplied to aquariums from an air blower. The shrimps were fed two times per day (5-7 % total shrimp weight) with commercial food (camaronina) supplied by Purina® of Mexico. The aquariums water was changed three times a week. Every time water was changed, 3 ml of each experimental PAHs dissolved in acetone were added to aquariums. To determine the sublethal concentration of PAHs, several concentrations were assayed, until zero shrimp mortality was obtained. So, experimental concentrations of each PAHs in aquariums water, were the following: Pyrene (1.3 µg/l), Phenanthrene (3.3 µg/l), Naphthalene (2.6 µg/l), Chrysene (1.6 µg/l), Anthracene (4.2 µg/l), Fluorene (12.5 µg/l), Fluoranthene (5.2 µg/l), Benzo (b) fluoranthene (3.5 µg/l) and Benzo(a)pyrene (1.5 µg/l). As control, just 3 ml of acetone was added to aquarium water. At the end of exposure time (21 days), 5 shrimps from each aquarium were measured and weighed; then accord to the procedure of [22] with some modifications, around 50 µl haemolymph was

extracted from each one shrimp, using a 0.5 ml micro-syringe, previously impregnated with a 5 % EDTA solution as anticoagulant; after, 25 µl haemolymph of each shrimp was spread on slides covered with a thin layer of Agarose MMT (medium melting temperature), and a second Agarose layer LMT (low melting temperature) was added to slides. After solidification, the slides were Immersed in Alkaline Unwinding Solution (200 mM NaOH, 1 mM EDTA) pH>13 during 60 min at 4 °C in the dark. Subsequently, the slides were placed in a horizontal electrophoresis chamber, and then covered with a pH ≥8 electrophoresis buffer (10 mM Tris, 1 mM EDTA) and an electric strength of 300 mA and 25 V was applied using an electrophoresis power source BIORAD® FB-300 by 15 min. The slides were taken off from electrophoresis tray, immersed in distilled water for 5 min, and then in 70% ethanol during 5-6 min, air dried and stained with (0.001 µg/ml) ethidium bromide (EB) by 30 min at room temperature in the dark. All the reagents were purchased in Sigma-Aldrich® de Mexico. Mexico City. Slides were rinsed in distilled water to discard excess of EB and dried at room temperature. In order to assess the genotoxic damaged by PAHs to shrimp haemocytes, the slides were observed by fluorescence microscopy at 496 nm/522 nm for maximum excitation/emission, using a Leitz Laborlux S Fluorescence Microscope coupled to a digital camera Leica DFC-490 of 8 Mega Pixels, and a Dell monitor. The comet assay parameters tail length (TL), tail intensity (TI) or percentage of DNA in tail, and tail moment (TM) understood as tail length multiplied by the DNA fraction in the tail, were assessed using the software (Comet Assay IV®) supplied by Instem Co. Staffordshire, UK. Also, the amount of micronucleus in shrimp haemocytes were quantified by counting them in several microscopic fields, after an Eosin staining of haemocytes smears. Once finalized the genetic tests, the growth of exposed shrimp was estimated, by quantifying the weight increase of shrimp at end of exposure time.

A. Statistical analyses

All laboratory experiments and water analysis were performed by triplicate. The statistical parameter Mean and Standard Deviation (SD) of water samples, and growth experiment were determined by Excel of Microsoft Office 2010. The TL (µm), TM and TI (%) parameters are reported as the Mean and SD of each PAHs used in genotoxic experiments. Analysis of variance (ANOVA) and Newman-Keuls tests, were used to determine significant differences between experimental PAHs and control, using a GraphPad Prism software. A significance was defined as $p \leq 0.05$.

III. Results And Discussion

A. PAHs in water from ecosystems studied

From the 16 priority PAHs stablished by [21] only 9 were found in water samples of both studied ecosystems. Their mean value concentrations in µg /l, are presented in Table I. As can be observed, Chrysene was the most frequent congener found with 79.2%, and also at higher concentration, followed by Phenanthrene with 62.5% and Fluorene with 50%. The others congener ranged from 45.8 to 25 %, and at lower concentrations than studied ecosystems. On the other hand, the higher concentrations were registered during rainy season.

Although concentrations of PAHs found in the studied ecosystems were lower than values reported by US coastal water [13] and coastal systems of other countries [20], in some cases the values were similar or higher than concentration used in the aquariums of the experimental assays; such as sub-lethal concentrations of Chrysene and Pyrene.

On the other hand, other authors, reported that PAHs are characterized by their hydrophobic properties, which allow them be adsorbed with suspended particulate matter and finally deposited in the sediment, which constitutes a PAHs reservoir; therefore, sediments become an important source of pollutants and can be a significant risk to aquatic organisms [23]. Also, there is a dynamic PAHs transfer between water and sediments; consequently, compounds adsorbed in sediments such as PAHs, becomes easily available to benthic organisms such as shrimp, crabs, clams, etc. [24].

B. Genotoxicity and chromosomal damage

The results of comet assay parameters are shown in Table II. For practical purposes, from all the samples processed, only the results of 22 samples, randomly selected are presented; otherwise, the amount of data in tables would be too large. As can see, the values presented indicate that PAHs genotoxicity to shrimp, is evident. There is not a clear correlation between parameter values of comet assay, and exposure concentrations of PAHs in aquarium water, presented as (Sample Code) in Table 2. This can be due that shrimp in laboratory experiments, were exposed at sub-lethal concentrations, i.e., the experiments objective, was not to know genotoxicity vs. PAHs concentrations, such that has been, in other works [22]. However, the DNA damage (strand breaks) was similar or lower than values reported by other authors, where crustaceous or other estuarine invertebrates, were exposure to some PAHs [25], [26], [27].

The results of the micronucleus frequency (index of chromosomal damage) of shrimp haemocytes are shown in Table III. The data demonstrate that PAHs are causing chromosomal damage in shrimp exposed to these pollutants, since the micronucleus frequently was increased. Therefore, results shown in Table II and Table III, indicate environmental impact by human activities such as boat´s transit, accidental fuel spilling and other activities, which spill petroleum derivate in coastal ecosystems studied in this work. On the other hand, results demonstrate that juvenile shrimps (*Litopenaeus vannamei*), can be used as bioindicator of aquatic pollution; and the comet assay and micronucleus frequency, as powerful tools for the assessment of DNA genotoxicity, and chromosomal damage to shrimp haemocytes by PAHs.

TABLE I: CONCENTRATION IN (μG/L) OF PAHS IN WATER SAMPLES OF STUDIED COASTAL SYSTEMS. ABBREVIATIONS: NA: NAPHTHALENE; FLU: FLUORENE; PHE: PHENANTHRENE; ANT: ANTHRACENE; FLUO: FLUORANTHEN; PYR: PYRENE; CHRY: CHRYSENE; B(B)F: BENZO(B)FLUORANTHEN; B(A)P: BENZO(A)PYRENE. ND: NO DETECTED; H-C: HUIZACHE-CAIMANERO LAGOON; TEA: TEACAPAN ESTUARY

Sampling data	Na	Flu	Phe	Ant	Fluo	Pyr	Chry	B(a)P	B(b)F
H-C.1/Jan-2019	ND	ND	ND	ND	ND	ND	0.17 ±0.014	ND	ND
H-C.2/Jan-2019	ND	ND	0.041±0.005	ND	ND	ND	ND	ND	ND
H-C.3/Jan-2019	0.038±0.004	0.031±0.003	0.029±0.0022	ND	0.023±0.0021	ND	0.245±0.023	0.019±0.0015	ND
Tea.1/Jan-2019	ND	ND	ND	0.031±0.009	ND	ND	ND	ND	ND
Tea.2/Jan-2019	0.033±0.008	0.022±0.008	ND	ND	ND	ND	0.314±0.049	ND	ND
Tea.3/Jan-2019	ND	ND	0.042±0.009	ND	ND	ND	ND	ND	ND
H-C1/ .Mar-2019	ND	ND	ND	0.028±0.004	ND	ND	0.59±0.12	0.021±0-0017	ND
H-C.2/ Mar-2019	ND	0.031±0.007	0.029±0.003	ND	ND	ND	0.93±0.087	ND	ND
H-C.3/ Mar-2019	0.037±0.007	ND	0.037±0.004	ND	0.028±0.004	0.025±0.002	1.65±0.24	0.018±0.0015	ND
Tea.1/Mar-2019	ND	ND	ND	0.035±0.003	ND	ND	ND	ND	ND
Tea.2/Mar-2019	0.027±0.006	ND	ND	ND	ND	ND	2.34±0.32	ND	ND
Tea.3/Mar-2019	ND	0.037±0.008	ND	ND	ND	ND	2.72±0.15	ND	0.036±0.005
H-C.1/Jul-2019	ND	0.065±0.005	0.061±0.007	0.063±0.009	ND	0.051±0.004	6.74±0.62	0.043±0.004	ND
H-C.2/Jul-2019	ND	0.068±0.009	ND	ND	ND	0.035±0.004	5.53±0.68	ND	ND
H-C.3/Jul-2019	0.67±0.15	ND	0.132±0.058	ND	0.066±0.008	0.064±0-008	10.45±1.02	0.047±0.007	0.058±0.005
Tea.1/Jul-2019	0.818±0.089	ND	0.135±0.048	ND	ND	0.122±0.095	6.87±0.92	0.051±0.006	ND
Tea.2/Jul-2019	ND	0.151±0.012	0.119±0.09	0.059±=0.009	0.091±0.099	0.088±0.006	16.14±1.54	ND	ND
Tea.3/Jul-2019	0.79±0.067	0.179±0.021	0.127±0.041	ND	ND	0.127±0.011	4.42±0.42	ND	0.062±0.012
H-C.1/ Sep-2019	0.66±0.057	0.137±0.017	0.125±0.012	0.083±0.018	0.131±0.011	ND	10.59±0.49	0.073±0.008	
H-C.2/ Sep-2019	ND	ND	0.132±0.03	ND	ND	1.42±0.16	7.93±0.77	ND	0.078±0.072
H-C.3/ Sep-2019	0.75±0.069	0.147±0.015	ND	0.088±=0.009	ND	1.25±0.32	9.65±0.89	0.064±0.007	0.068±0.011
Tea.1/Sep-2019	ND	0.165±0.016	0.171±0.034	0.135±0.019	0.098±0.009	ND	ND	0.073±0.007	ND
Tea.2/Sep-2019	0.75±0.068	ND	0.158±0.016	0.108±0.061	ND	1.67±0.31	15.34±1.42	0.098±0.014	0.089±0.012
Tea.3/Sep-2019	0.74±0.071	0.162±0.018	0.131±0.015	0.122±0.057	ND	1.49±0.26	8.72±0.74	0.068±0.005	ND

In the following figures, are shown the photographs by fluorescence microscopy of haemocytes cells of shrimps exposed to sub-lethal concentrations of Chrysene (1.6 μg/l), Pyrene (1.3 μg/l), Phenanthrene (3.3 μg/l), Naphthalene (2.6 μg/l),) Fluorene (12.5 μg/l), Anthracene (4.2 μg/l), Fluoranthene (5.2 μg/l), Benzo(b)fluoranthen (3.5 μg/l), Benzo(a)pyrene (1.5 μg/l) and control (3 ml) of acetone. As can be seen the comets in haemocytes were present in all shrimp exposure to PAHs, except in control.

The comets of haemocytes cells in above photographs (Figs 2 to 10) indicate that PAHs are genotoxic substances to shrimp even at low concentration; however, is necessary to consider that in the natural habitat, surely there are other xenobiotic compounds which could cause damage to DNA, mutagenicity and other toxicological alterations; therefore, laboratory experiments have a relevant value, but cannot be considered absolute. Other authors agree with this premise, since they consider that it is important to be careful with risk measures, because it is necessary to understand how the biota behaves in its totality, which implies a considerable different between laboratory results using model organisms, with the real conditions in their habitat [28].

Regarding the growth of exposed shrimp, a decrease in weight of all shrimp exposed to PAHs was detected, Fig. 12. As can be seen, the greatest decrease in weight correspond to shrimps exposed to Chrysene, whereas the smallest to Anthracene. This can be due to shrimp under toxic stress has a higher energy consumption to keep their vital functions; therefore, the energy available to growth becomes reduced; also, due to some features of this pollutants, since once the PAHs has been absorbed into organs and tissues of shrimp, they are distributed and accumulated, mainly in lipophilic tissues, and then, can be bio-transformed in other compounds by oxy-reduction reactions catalyzed by cytochrome P450, mixed function oxygenases, NADPH-cytochrome, as well by conjugation reactions catalyzed by

glutathione-S-transferase, sulfotransferase, epoxide hydrolase, and other transferase enzyme, increasing its water solubility [29]. The products of all these reactions can generate compounds with higher toxicological activity, causing genetic damages to aquatic organism, particularly to benthonic such as shrimp, since in bottoms of aquatic systems, all these reactions are more fast due to the benthic organisms are in intimal contact with the bottom. Other authors arrive to similar conclusions [30].

TABLE II: TAIL INTENSITY, TAIL LENGTH AND TAIL MOMENT IN HAEMOCYTES CELLS OF SHRIMP EXPOSED TO SUBSETAL PAHS CONCENTRATIONS IN LABORATORY EXPERIMENTS.

Sample Code	Tail intensity (% of DNA in in tail) Mean ± SD	Tail length Mean ± SD	Tail oment Mean ± SD	PAH congener
CICON	8.0 ± 1.52	24.44 ± 1.02	0.94± 1.58	Naphthalene
C2CON	10.0 ± 2.23	25.99 ± 1.20	1.55 ± 0.56	Fluorene
C3CON	16.0 ± 23.40	16.32 ± 1.22	1.44 ± 1.02	Anthracene
C2MON	5.0 ± 1.44	19.56 ± 1.78	0.91 ± 1.3	Chrysene
C6PO1	12.0 ± 1.31	21.41 ± 1.08	0.94 ± 0.64	Pyrene
C2PO1	6.0 ± 1.38	18.99 ± 1.88	1.58 ± 0.77	Naphthalene
C4PO1	10.0 ± 1.23	15.99 ± 1.20	1.44 ± 1.88	Phenanthrene
C3PO1	16.0 ± 1.66	19.33 ± 0.93	1.71 ± 1.65	Fluorene
C1MON	12.0 ± 2.1	25.44 ± 1.10	1.25 ± 1.55	Naphthalene
C3MON	18.0 ± 1.77	16.89 ± 1.23	1.86 ± 2.65	Benzo(a)pyrene
C4MON	15.0 ± 0.97	22.63 ± 2.28	0. 77 ± 1.02	Anthracene
C5MON	18.0 ± 1.12	24.44 ± 1.02	1.06± 0.71	Chrysene
C7CON	18.0 ± 1.02	15.14 ± 1.02	0.99± 0.44	Fluorene
C6MON	16.32 ± 0.92	18.40 ± 1.02	1.09±1.21	Chrysene
C7MON	10.0 ± 1.25	21.99 ± 1.20	1.33 ± 0.70	Phenanthrene
C1PO1	14.44 ± 1.02	26.32 ± 1.22	1.69 ± 1.02	Pyrene
C4CON	16.32 ± 1.22	19.11 ± 1.78	0.91 ± 1.31	Pyrene
C5CON	19.56 ± 1.78	51.41 ± 0.98	1.86 ± 1.64	Chrysene
C6CON	15.0 ± 1.07	52.63 ± 1.30	1. 77 ± 0.22	Phenanthrene
C7CON	10.0 ± 2.00	17.79 ± 1.20	1.64 ± 8.68*	Naphthalene
C7POI	10.0 ± 1.03	15.99 ± 1.20	1.44 ± 0.88*	Anthracene
C5POI	16.32 ± 1.22	16.97 1.78	0.81±1.33	Benzo(a)pyrene

*Number of haemocytus quantified was ≤ 1000.

TABLE III: MICRONUCLEUS FREQUENCY OF HAEMOCYTES CELLS OF SHRIMP EXPOSED TO EXPERIMENTAL PAHS

Sample Code	Micronucleus frequency	PAH congener
CICON	1.0	Naphthalene
C2CON	2.0	Fluorene
C3CON	1.0	Anthracene
C2MON	1.0	Chrysene
C6PO1	2.0	Pyrene
C2PO1	1.0	Naphthalene
C4PO1	1.0	Phenanthrene
C3PO1	2.0	Fluorene
C1MON	1.0	Naphthalene
C3MON	1.0	Benzo(a)pyrene
C4MON	2.0	Anthracene
C5MON	1.0	Chrysene
C7CON	1.0*	Fluorene
C6MON	2.0	Chrysene
C7MON	1.0*	Phenanthrene
C1PO1	3.0	Pyrene
C4CON	1.0	Pyrene
C5CON	2.0	Chrysene
C6CON	1.0*	Phenanthrene
C7CON	2.0	Naphthalene
C7POI	3.0	Anthracene
C5POI	2.0	Benzo(a)pyrene

* Micronucleus Frequency quantified in haemocytus was ≤ 1000.

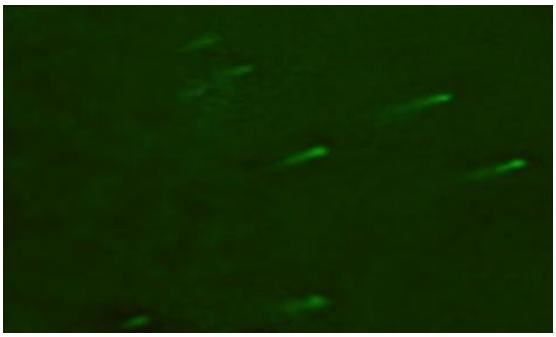

Fig 2. Nucleus of haemocytes cells of shrimp exposed to Chrysene, after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

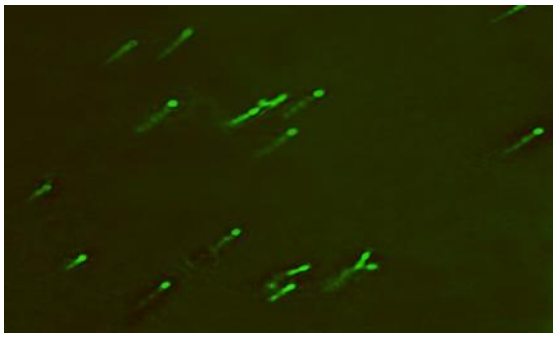

Fig 3. Nucleus of haemocytes cells of shrimp exposed to Pyrene, after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

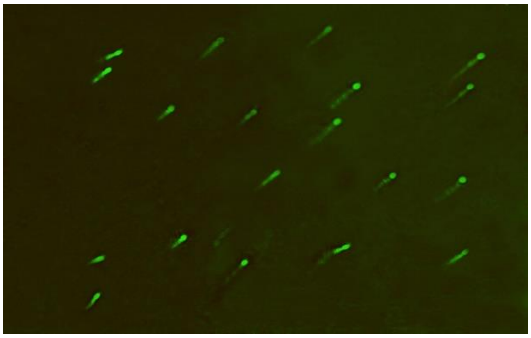

Fig 4. Nucleus of haemocytes cells of shrimp exposed to Phenanthrene, after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

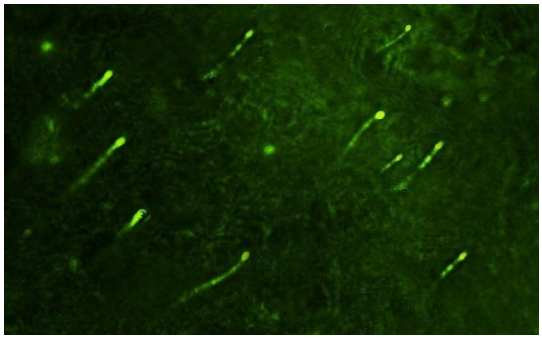

Fig 5. Nucleus of hemocytes cells of shrimp exposed to Naphthalene, after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

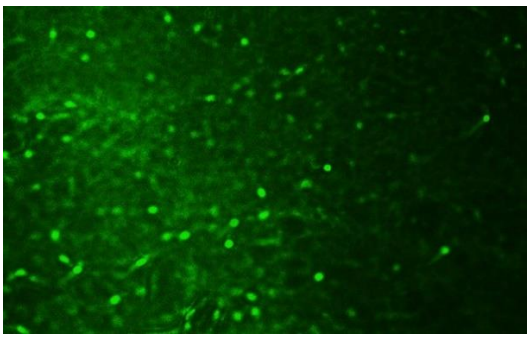

Fig 6. Nucleus of hemocytes cells of shrimp exposed to Fluorene, after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

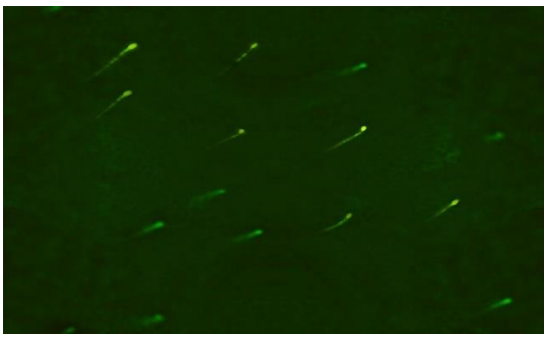

Fig 7. Nucleus of hemocytes cells of shrimp exposed to Anthracene , after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

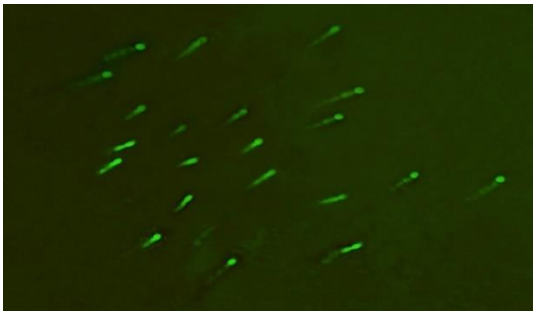

Fig 8. Nucleus of hemocytes cells of shrimp exposed to Fluoranthen , after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

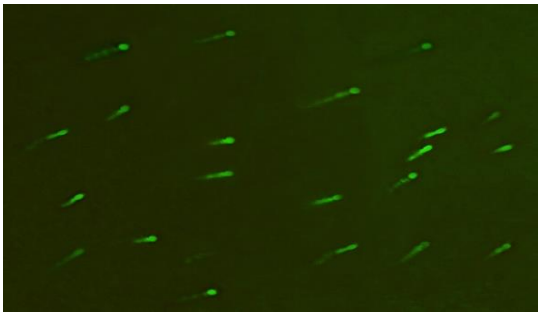

Fig 9. Nucleus of hemocytes cells of shrimp exposed to Benzo(b)fluoranthen, after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

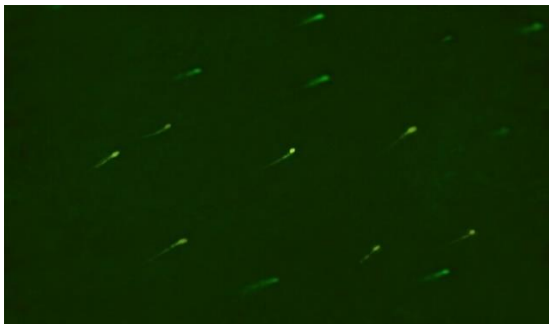

Fig 10. Nucleus of hemocytes cells of shrimp exposed to Benzo(a)pyrene, after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

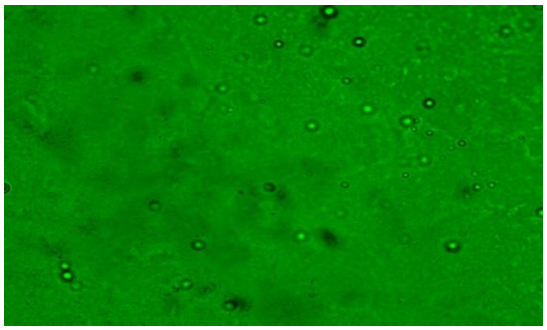

Fig 11. Nucleus of hemocytes cells of shrimp exposed to 3 ml of acetone (control), after the comet assay procedure, stained with 0.001 µg/ml ethidium bromide, and observed at 40X in a fluorescence microscope.

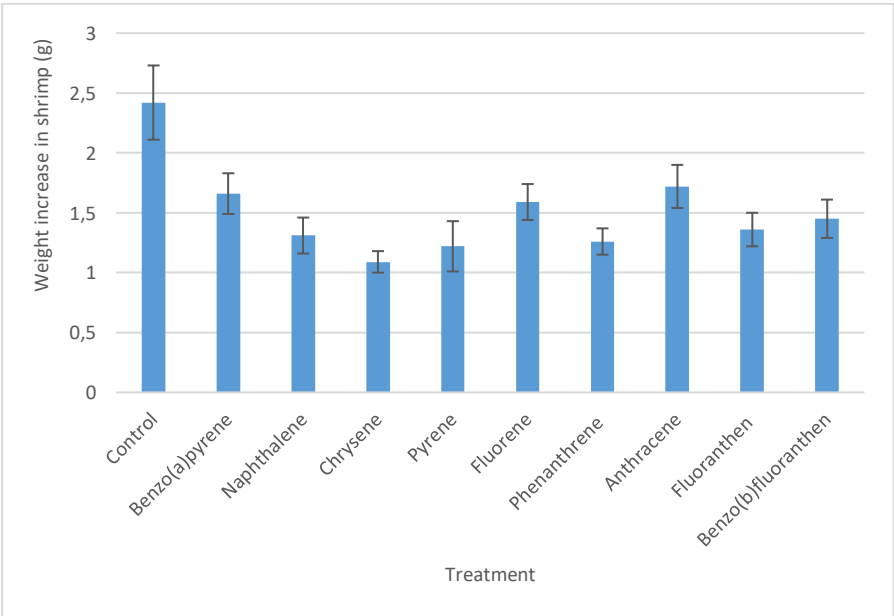

Fig. 12. Weight Increase in shrimp exposed to PAHs during 3 weeks. As can be seen the Chrysene was the congener that caused the lowest growth in shrimp.

IV. Conclusion

From PAHs concentration in water of coastal ecosystems studied, it is possible to conclude that they are relatively low compared to values reported for some coastal ecosystems of the US and other countries. However, these concentrations are sufficient to cause genotoxic damage to shrimp and possible infectious diseases; and also, a decrease in its growth, generating considerable losses in shrimp aquaculture. Furthermore, since these contaminants can bioaccumulate in the tissues of many aquatic organisms, the HAPs pollution becomes a risk to human health by consuming these seafood. Consequently, to reduce the amount of PAHs and decrease shrimp aquaculture losses; Production methods, currently used in agriculture and also in shrimp aquaculture, should be changed to more sustainable methods.

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