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Pollution with Polycyclic Aromatics Hydrocarbons (PAHs) in *Lutjanus synagris* and *Centropomus undecimalis* Coming from the Gulf of Morrosquillo, North of Colombia

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Abstract : Samples of fish species Lutjanus synagris (lane snapper, pargo biajaiba or pargo rayado) and Centropomus undecimalis (Common snook, sergeant fish or robolo) from municipality of Covenas and corregimiento of Berruga in the Gulf of the Morrosquillo (Department of Sucre, northern Colombia), were collected to evalute therir pollution degree with polinuclear aromatic Hydrocarbons (PAHs), also called Polycyclic Aromatic Hydrocarbons. Analysis to detect residual concentrations of siteen posible PAHs in fish muscles were carried out with gas chromatography coupled by masses (GC/MS). Results showed 11,230 $\mu g.kg^{-1}$ of PAH saverage total concentrations for Lutjanus Synagris (ranging from 0,004 to 145,596 $\mu g.kg^{-1}$) and 8,596 $\mu g.kg^{-1}$ for Centropomus undecimalis (ranging from 0,002 to 85,915 $\mu g.kg^{-1}$) where PAHs classified as carcinogenic (venzo[a] pyrene, dibenzo [a, h] anthracene), showed a low frequency in the detection. This condition allows to consider that PAHsaverage values do not represent an immediate risk for human health, but it would be a potential one because of the bio-accumulation property of these pollutants. Thus, a continuous monitoring of this problema is recommended since it could become a risk for the environment and public health.
Keywords : Gulf of Morrosquillo, Aromatic hydrocarbon, Pollution, Fish, Bioaccumulation.

Introduction

The Gulf of Morrosquillo, in northern Colombia, is an area of high tourist significancedue to its excessive beauty and food source for the Sucre Department and the entire region. That's why this area has great fishing potential, both for development of inland fisheries and for maritime fishing, since 15% of the territory are water bodies and it has 102 kilometers of coastline, including 45 kilometers from the Gulf.Main fishes found in these waters include: Atlantic Horse Mackerel, Snoek, Snapper, Bar jack, Common Snook, Anchovy, and others, making it an area of great hydrobiological richness[1].

Nonetheless, this territory has faced different contamination events during the last years due to crude oil accidental spills from the port of Covenas (a terminal from the *Caño Limón* - Covenas Pipeline District, used to discharge oil).According to reports from the department environmental authority CARSUCRE[2], through press release, the closest event was an incident occurred on October 21, 2017 during the loading maneuvers of tanker

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"Anne" when there was a leak from one of the floating hoses with approximately 13 barrels of oil that formed a 3.2-kilometer slick in the Tolu municipality and another 1.8 kilometers on *La Caimanera* Swamp. According to the same release, it was possible to recover approximately 90% of spilled crudewith floating barriers. Although hydrocarbon spills are common during the production, loading and distribution processes, they danger the soil, water, fauna, fish resources, flora and even to human health.

PAHs are included among products or substances associated with crude oil. Many of which carcinogenic and toxic to living organisms. Additionally, due to their properties, they can bioaccumulate in fatty tissues of aquatic organisms, which are key compounds for the determination of pollution in marine ecosystems. As a complement, and important data for the present research, the study area (Municipality of Covenas) has been classified as a vulnerable area at the national level, due to the environmental effects that could occur on the natural resources (fishing and mangrove) which mostly represent the basis for the food security of the region's[3,4,5].

Within the Gulf, for the specific cases of Santiago de Tolu and Coveñasbeaches, with a high tourist activity, the main established sources of pollution to the sea are: 1. Inadequate disposal of solids and poor handling of liquid discharges to the sea, and 2. The typical economic activities of the region exertive excessive pressure on the coastal lane such as fishing, maritime transport and tourism. These cause alteration of marine waters natural properties as they favor the rise of concentrations of petroleum hydrocarbon residues, nutrients and organic matter[6].This highlights the worth of this research where pollution degree with PAHs in commercial species such as *Lutjanus synagris* and *Centropomus undecimalis* from the Gulf of Morrosquillo were evaluated, specifically in the Coveñas municipality and *corregimiento* of Berrugas, department of Sucre - Colombia.

Materials and Methods.

Type and Study Location

This is a descriptive research and conducted in the Gulf of Morrosquillo, covering the municipalities of Coveñas (09 ° 24 '00 N latitude, 75 ° 40' 55 E longitude; a high exposure area to the spills) and San Onofre, specifically *corregimiento* of Berrugas (9 ° 41 '08 "latitude N; 75 ° 36 29" longitudeW; Area of least exposure to spills) in the Sucre department. (Figure 1).

Figure 1. Gulf of Morrosquillo. Sources: http://www.imeditores.com/banocc/golfos/mapas

Study Area and Sampling

4 samplings of 10 specimens of fish under study were conducted for six months in the municipality of Coveñas and *corregimiento* of Berrugas, in the sites referenced in Figure 2 and Table 1. Fish capture was done by fishermen in the area with traditional cast nets. Specimens were stored at 4 ºC and transported to laboratory for further hydrocarbons analysis.

Figure 2. Fish and sediment sampling points. A: Coveñas. B: *Corregimiento* of Berrugas. Modified from Google Earth, 2016

Table 1 Coordinates of the Sampling Locations

Location	**1**	**2**	**3**	**4**	**5**	**6**
Port of de Coveñas	9°24’54,36"	9°24’59,04"	75°41’15,80"	75°41’20,25"	75°41’23,04"	75°41’24,62"
	75°41’24,43"	75°41’21,69"	9°25’26,53"	9°25’16,51"	9°25’9,11"	9°24’57,99"
Berrugas	9°41’27,37"	9°41’21,53"	75°36’57,52"	75°36’54,24"	75°36’53,53"	75°36’52,55"
	75°36’51,17"	75°36’56,60"	9°41’12,91"	9°41’17,37"	9°41’20,93"	9°41’23,76"

Hydrocarbon analysis (PAHs)

Subsamples of muscles for aromatic polycyclic hydrocarbons analysis were obtained from the dorsal muscle using steel knives, cutting approximately 30 to 50g of each specimen and depositing them in aluminum bags. They were lyophilized at -40°C and 0.13 vacuum mbar,in the Laboratory,using a Labconco lyophilizer. For PAHs analysis, 50 g of muscle were taken and subject to solid-liquid extraction using Boodtubes for 16 hours, with dichloromethane as solvent. Result products were concentrated in a rotavapor until getting an approximate volume of2mL. Purification was done through application of a Florisil column up toa1ml volume concentration. This solution was analyzed through gas chromatography with mass spectrometer detection (GC / MS) to determine concentration[7].

Statistical analysis.

Results of this study are presented as mean ± standard error for all statistical analyzes implying means comparison (t-test and performing of ANOVAS with tukey posttest). Bartlett test of variance homogeneity and normality tests (Kolmogorov-Smirnov) were initially applied.

An ANOVA with Post Sum of Squares type III was performed to compare mean concentrations of PAHs in the respective fishes and sediment samples after verification of homogeneity and normality of variance.

Results

Table 2 shows PAHs concentrations obtained in the analysis of the two-fish species studied, according to place of origin. Different compounds analyzed include: Acenaphthene, Naphthalene, Phenanthrene, Pyrene and Benzo (a) pyrene, all listed as priorities according to the Environmental Protection Agency (EPA)[8].

Table 2 PAHs Concentration of in fish species of the Gulf of Morrosquillo.

PAHs / Location		PAHs Concentrations (ng/g)				
		Acenaphtene	Naphtalene	Phenantrene	Benzo (a)pyrene	Pyrene
Coveñas	Snook (1)	2,13 ± 1,42	80421,6±30211,3	1,32±0,495	ND	0,67±0,24
	Lane Snapper (2)	3,97± 2,21	14825,3±9491,2	188,57±103,07	61,07±40,72	61,3±28,60
Berrugas	Snook	ND	91408,4±26548,9	15,75±4,28	ND	12,87±4,89
	Lane Snapper	3,10±1,70	14293,8±5771,6	100,04±80,56	99,37±42,70	43,09 ±20,50

(1) *Centropomus undecimalis* (2) *Lutjanus synagris ND*: **No detectable.**

The study confirmed PAHs presence in Snook and Lane Snapper, both in *corregimiento* of Berrugas and municipality of Coveñas. Naphthalene was the compound with the highest concentration in the analyzed fish samples. Likewise, research results showed that there are no statistically significant differences between concentrations means of the compounds evaluated, with a 95.0%confidence level.

On the other hand, in the box plotsfrom figure 3, by comparing fish species evaluated; lane snapper is the species reflecting the greatest variability in concentrations of polluting residues of PAHs; more specifically of compounds such as Acenaphthene and Benzo (a) pyrene.

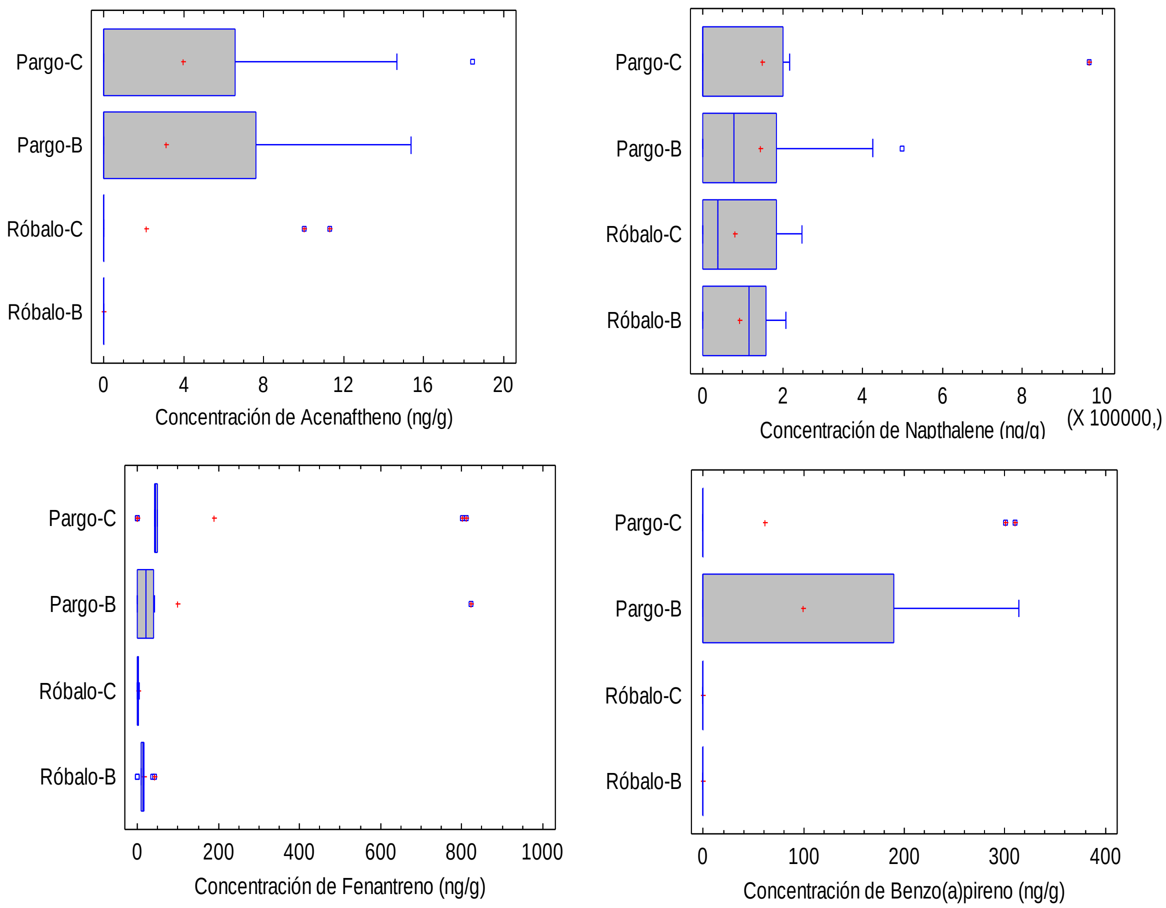

Figure 3. Variability of PAHs in fish species from Gulf of Morrosquillo. Snapper C (Coveñas) Snapper B (Rugs) Snook C (Coveñas) Snook B (Berrugas).

The following graph (Figure 4) shows 4 of the main hydrocarbons obtained, corresponding with that found by Bonert, *et al* (2010)[9], who show the presence of three PAHs of low molar mass (LPAHs): naphthalene, phenanthrene and anthracene (2 and 3 rings) and three of high molar mass (HPAHs): fluoranthene, pyrene and benzo (b) fluoranthene (4 and 5 rings).

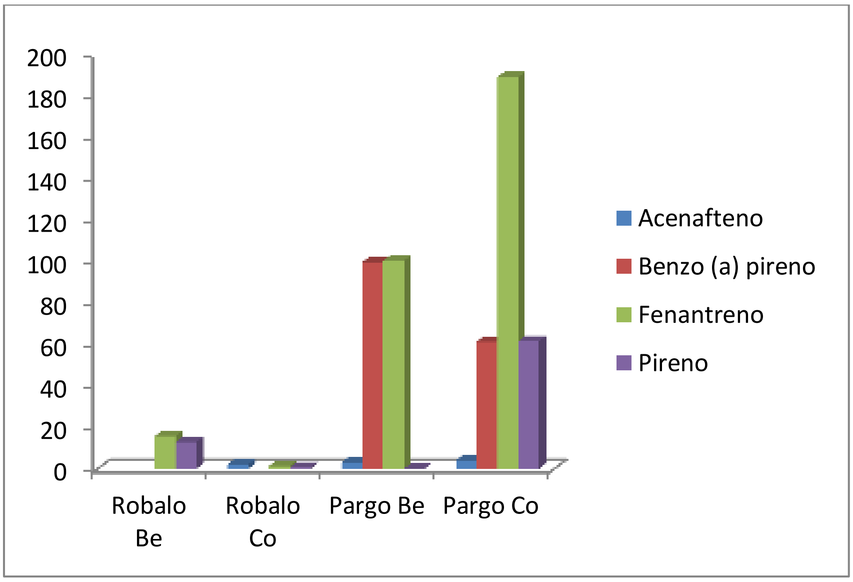

Figure 4 Concentration of Polycyclic Aromatic Hydrocarbons in fishes from Coveñas (Co) and Berrugas (Be).

Considering that there are two sampling sites with their own characteristics and different environmental pressures, an ANOVA was performed, shown in Table 3.

Table 3. Analysis of variance to determine influence of the site of origin and the species in the concentrations of PAHs presented in fish muscle.

No	Compound	P-Value Location	P-Value Species
1	Naphthalene	0,8818	0,0043*
2	Acenaphtne	0,9687	0,1165
3	Fenanthrene	0,8177	0,0058*
4	Pyrene	0,1774	0,0012*
5	Benzo (a) pyrene	0,0035*	0,0150*

* Indicates a significant difference

Finally, to determine the relationship between the species under study and sampling sites, the correlations are performed.The results are shown in Tables 4 and 5 for Coveñas and Berrugas respectively.

Table 3 Correlations Coveñas

Fishes/Compounds		Naphtalene	Acenaphtene	Phenantrene	Pyrene	Benzo[a]pyrene
LANE SNAPPER	R2	-0,3306	-0,2112	-0,2414	-0,3741	-0,6429
	P	0,3507	0,5581	0,5016	0,2869	0,045*
SNOOK	R2	0,0094	-0,1207	-0,431	0,049	---
	P	0,9824	0,7759	0,2863	0,9082	---

Table 4 Correlations Berrugas

Fishes/Compounds	Naphtalene	Acenaphtene	Phenantrene	Pyrene	Benzo[a]pyrene

LANE SNAPPER	R2	-0,5769	-0,4908	-0,3499	-0,5496	-0,5975
	P	0,0808	0,1498	0,3217	0,0998	0,0681
SNOOK	R2	0,5645	----	0,159	-0,0447	----
	P	0,0891	----	0,6609	0,9024	----

Discussion

As previously mentioned, oil spills are common during crude production processes, as well as during commercialization, transportation and storage at collection and distribution sites. Massive and continuous contribution from various tasks are added to this type of pollution related to petroleum-derived substances, such as: fishing; maritime transport, ship cleaning operations, fuel sales to small boats, waste dumping for oil changes and lubricant cans in boats; all these anthropic activities accumulate with time, and little by little generate serious environmental impacts for aquatic ecosystems.

When hydrocarbons pollute shallow water bodies, they tend to float due to density difference presented with respect to water. Thus, they block light penetration and gas exchange, favoring solubilization of materials that affect different populations such as phytoplankton, micro-invertebrates and fishes. Correct development of photosynthesis is also prevented. Thus, direct affectation on fauna and flora developed in such an ecosystem is evident[10].

Research reveals that PAHs toxic effects depend on concentrations and exposure time of the aromatic components they possess. This way, natural populations suffer acute toxic effects after large PAHs spills, where the direct responsible are aromatic components with low molecular weight,corresponding with the compounds found in both species of study (anthracene, naphthalene and phenanthrene). In addition, these types of PAHs can be dispersed in water and confined in an intertidal or low depth manner, which implies analyzing that there are fish species more vulnerable to being contaminated than others. The fish species of the present study are clear examples of the above because they are demersal species with a wide distribution in the Caribbean region. *Lutjanus synagris* is of shallow bottomshabits, such as soft bottoms, rocky and coral substrates,[11 12,] and *Centropomus undecimalis*, is a species that frequently interacts in estuarine zones.In the gulf of Morrosquillo, the estuarine ecosystem 'Caimanera marsh'can be identified and located 6 km approximately to the terminal of Coveñas.

With respect to methodological difficulties arisen in the research on PAHs toxic effects, recent laboratory studies have shown and reported that species exposure to low concentrations of components with high hydrocarbon toxicity can cause deterioration of diverse physiological functions, such as breathing, movement, and reproduction. Also, likelihood of genetic mutations in eggs and larvaemay increase. Nonetheless, it is difficult to detect these sublethal effects in the natural environment. Despite the mortality of eggs and larvae that could occur after a spill, there is rarely a subsequent decrease in natural adult population. This can be explained in part by the considerable natural recovery of marine ecosystems to various high-gravity impacts.Marine organisms adapt rapidly to naturally high mortality levels, among other reasons by producing large surpluses of eggs, larvae andincorporation of populations reserves outside the affected area[13].

The hydrocarbons: Acenaphthene, Naphthalene, Phenanthrene, Pyrene and Benzo (a) pyrene found in *Lutjanus synagris* (lane snapper) and *Centropomus undecimalis* (snook) from Coveñas municipality and *corregimiento* of Berrugas in the Gulf of Morrosquillo correspondtowhat was found by Bonert, *et al* (2010)[9].Showing presence of three PAHs of low molar mass (LPAHs): naphthalene, phenanthrene and anthracene (2 and 3 rings) and three of high molar mass (HPAHs): fluoranthene, pyrene and benzo (b) Fluoranthene (4 and 5 rings).

Naphthalene can be identified as a modestly volatile compound, which allows its concentration to decrease more easily in the environment versus other compounds of the same group.This characteristic is related to its low molecular weight (MW) which in turn allows it to be available to organisms facilitating their mobility with respect to other PAHS and being retained for short periods of time[14]. Nonetheless, the compound with the highest concentration in the present study was naphthalene, corresponding with that reported by Pozo K. *et al*. (2011)[15], in that naphthalene, the simplest of the low molecular weight PAHs, was found at relatively elevated levels, which suggests that its source was mainly anthropogenic[16].

In the case of fluoranthene,its main characteristic is to be more persistent compared to the group lighter compounds and this accumulates in greater proportion with respect to them; as reported in evaluations carried out with other species such as mollusks.[17, 18, 19]inwhich fluoranthene and pyrene, were found for both sampling sites in all study species. These concentrations corresponded additionally with the study carried out by Llobet *et al*, in 2008[20] where it is indicated that the PAHs (benzo (a) pyrene, dibenzo (a, h) anthracene),classified as carcinogenic,showed a low frequency in their detection. It is important to note that Otero (2013) [21] evaluated the effect of phenanthrene (polycyclic aromatic hydrocarbon) on the growth of *Chlorella vulgaris* microalgae under laboratory conditions, concluding that microalgae growth can be negatively affected by exposure to higher nominal concentrations to 1 µg / l of phenanthrene, indicating the impact that this type of hydrocarbons can generate and the consequences they could have on the natural dynamics of marine ecosystems, especially in the areas of the present study. This situation could confirm the fact that average PAHs values do not represent an immediate risk to human health, but they do turn out to be a long-term potential one, given the bioaccumulation properties of this type of pollutants.

Results showed that only *species* has an influence on residual contamination of compounds Naphthalene, Phenanthrene and Pyrene, unlike the compound Benzo (a) pyrene, in which it showed that both factors (species and sampling site) have influence, which allowto infer that this is due to the *species* ecological dynamics, mainly, in relation to their eating habits. Finally, it was presented that neither of the two factors have an influence for acenaphthene, with a value of P => 0.05.

When making comparisons of each polluting PHA with the studied species and the sites of origin, it was observed that, only for Naphthalene, Pyrene and Phenanthrene, there are reports in all species for both sites. While for Acenaphthene and Benzo (a) pyrene, they are found mostly in the species captured in Berrugas. This fact could be related to the incidence of maritime traffic and the abundance in the environment of compounds formed by the union of 2, 3 and 4 benzene rings added to bioaccumulation processes due to pollution with hydrocarbons in the rise levels of the trophic network [6, 22]

In general, this study coincides with what was reported by Muñoz in 2010, [22] where presence of PAHs in viscera and muscles of fish collected in water bodies affected by oil activity in Shushufindi, Sucumbios, Ecuador was confirmed. However, INVEMAR in 2015[23], reports that concentrations of total aromatic hydrocarbons (TAH) determined in shallow water fluctuated in values range lower than the limit of detection (0.07) and 1.37 µg / L; without exceeding the 10 µg / L reference value considered of high risk for the aquatic ecosystem. The highest concentrations according to INVEMAR were obtained in the 2014dry season, in the Tolu - Coveñas area, in the Caño Pechelín (1.37 µg / L) and Caño Francés (1.24 µg / Lstations), and the in the Gulf site in front of Berrugas (0.67µg / L).

Based on the results presented above, everything could indicate that presence of PAHs in these marine ecosystems is influenced by transportation, refining and use of petroleum as well as informal fuel and vehicle washer shops in areas around the channels made by fishermen and inhabitants of the area, as well as the influence of maritime activities, as expressed by INVEMAR (2005 and 2016) [4, 6], where it states that the main sources of pollution and deterioration of waters in the basin of the Caribbean are the discharges of: municipal, industrial and agricultural origin and is enhanced by the discharge of oily waste from maritime and port activity, as well as oil activity, causing presence of organic toxins in the marine-coastal environments of the region. For all the above, PAHs presence in fish in the referenced area is one of the supports provided by this research to alert the food security of the surrounding and / or dependent populations of this gulf, who use this resource as a source of food and marketing.

Conclusions

This study confirms PAHs presence in fish muscles collected in both Berrugas and Coveñas, which is largely due to the activity of loading and discharging hydrocarbons. PAHs presence in samples of fish muscles is a matter of interest and at the same time worrisome, since it gives an alert about the nutritional security of neighboring populations, which use these as a basic resource for their food and economic sustenance.

Given the high capacity of living organisms and specifically fishes to store chemical substances in their organism from the environment, they turn out to be indicators of high importance for monitoring and identification of the effects of marine pollution. This can also indicate that their consumption can become a health problem for populations that feed on this resource, if preventive measures are not taken in the ecosystems affected by human activities.

Finally, it is recommended to carry out and complement the present study with participation of multidisciplinary teams that allow to evaluate health risks of the population consuming these fishes, that include blood and urine analysis for the evaluation of PAHs presentin consumers.

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