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PAH Levels in Bivalve Mollusks from the Mexican Subtropical Pacific

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Marine and coastal environments specially coastal lagoons receive a large variety of pollutants derived from anthropogenic activities (NOAA 1998). Rivers are one of the main transport mechanisms of these pollutants from terrestrial environments to the coasts (Witt 1995). On the other hand, the quality of aquatic environments would assessed through the analysis of organisms considered indicators of pollution, such as mussels and other bivalve mollusks (Pereira et al. 1992; Jaffé et al. 1995; Lauenstein 1995; Beliaeff et al. 1997).

Bivalve organisms can also be used as indicators to predict expure, effects, and susceptibility due to the presence of anthropogenic substances (Sericano et al. 1995; Cantillo et al. 1997; Schlenk 1999). They can bioaccumulate a large variety of pollutants in levels higher than those present in surrounding water or sediments, and their behavior can be recorded in short periods of time (Baumard et al. 1998; Solé et al. 2000).

The amount of organic compounds synthesized by humans are well above 1.8 millions and production of new compounds increases each year. Its global production is about of 100 to 200 million tons per year. These compounds include the PAH and can be introduced into aquatic environments through atmospheric transport (Page et al. 1999), sprays, water filtration into the ground, wastewater (Valerio et al. 2000) and pluvial discharges (Bidleman et al. 1990), which, once transported are subjected to transformation and bioaccumulation processes (Zhou et al. 1996).

The environmental importance of PAH resides in their mutagenic and carcinogenic properties, mainly benzo(a)pyrene (Grimmer 1993).

The objective of this study was to evaluate the baseline levels of PAH in coastal systems from the Mexican Subtropical Pacific by using the most abundant bivalve species of this region as biological indicators.

MATERIALS AND METHODS

The study area is located in the coastal zone of the Mexican Pacific, from 28°00'

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N latitude and 111°05' W longitude to 19°12' N latitude and 104°50' W longitude (Figure 1). Bivalves were sampled in 29 stations during May 1996, in the region from Guaymas, Sonora, to Puerto Vallarta, Jalisco, Mexico. In each station, bivalves were collected manually and packed in aluminum foil, maintained in refrigeration. All samples were oven dried (50°C) and then macerated and homogenate. The analytical procedure for extraction and purification of PAH was carried out by the method of UNEP/IAEA/FAO/IOC (1993). Each set of 5 samples was accompanied by a blank. Five grams of dried tissue was soxhlet extracted with methanol (250mL) for 8 hr, then added KOH 0.7*M* (20mL) and tridistilled water and refluxed for 2 hr more. The extract containing saturated and aromatic fractions was purified by adsorption chromatography using glass columns (30cm long) packed with alumina and silica gel. The elution for the fraction 1 (saturated) was made with hexane (20mL); and the fraction 2 (aromatics) was eluted with 30mL of hexane: methylene chloride (9:1). The eluates were evaporated in a rotary flask to near 10 mL and then evaporated to near 3 mL under N_2 flow.

Separation of PAH was made by means of a Hewlett Packard gas chromatograph model 5890 equipped with capillary fused silica column (30 m x 0.25 mm ID x 0.25μm bonded 5%-phenilmethylsilicone). The temperature was programmed from 30 – 300°C with an increase of 4°C /min. Helium was used as carrier gas (flow 1 mL/min). Quantification and identification of PAH was by means of a standard mixture ("Chemical Service" PPH-10M) with 16 PAH: naphtalene (NA), acenaphthylene (AC), acenaphthene (ACE), fluorene (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FLU), pyrene (PY), benzo(a)anthracene (BA), chrysene (CR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BP), indeno(1,2,3-cd)pyrene (IP), dibenzo(a,h)anthracene (DA), and benzo(g,h,i,)perylene (BPE). The limit of detection was 0.01μg/g and recovery yields were up to 90%. The analytical performance of the method employed was accredited by the participation of the laboratory in an international intercomparison exercise conducted by the International Atomic Energy Agency (IAEA-140) for petroleum hydrocarbons (1997).

RESULTS AND DISCUSSION

A total of 16 individual constituents of PAH were determined in the 29 bivalve samples, pertaining to five species: *Crassostrea palmula*, *Crassostrea corteziensis*, *Crassostrea gigas*, *Crassostrea iridescens* and *Mytella strigata*. Mean concentration of PAH each species (Table 1) was as follows: *C. iridescens* (6.91μ/g) > *C. palmula* (6.47μg/g) > *M. strigata* (3.44μg/g) > *C. corteziensis* (2.16μg/g) > *C. gigas* (0.75μg/g); statistical differences not significatives between species were observed ($p>0.05$).

Figure 2 shows the total concentrations of PAH (ΣPAH) in each site covered by this study. It can be observed that the highest level was found in *C. palmula* with 22.86 μg/g at 11 site located in Pichilingue Bay at South Baja California; followed by *M. strigata* (18.16μg/g) at 24 site belonging Mexcaltitan, Nayarit; *C.*

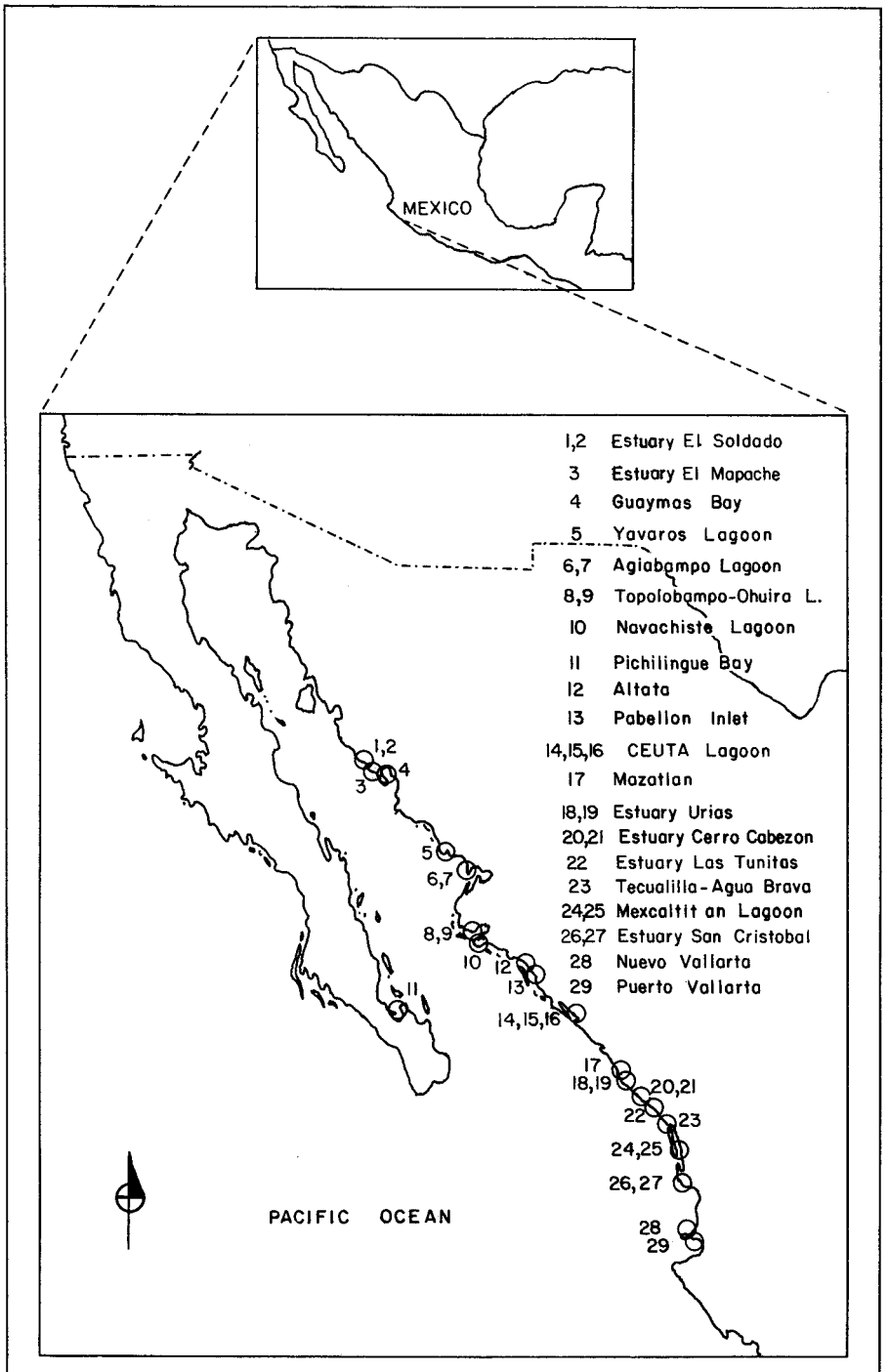

FIGURE 1. GEOGRAPHICAL LOCATIONS OF SAMPLING SITES.

Table 1.Mean concentrations of individual polycyclic aromatic hydrocarbons in bivalves from coastal systems of the Mexican Subtropical Pacific. ($\mu g g^{-1}$ dry weight)

COMPOUND	*Crassostrea palmula*	*Crassostrea corteziensis*	*Crassostrea gigas*	*Crassostrea iridescens*	*Mytella strigata*
COMPOUND AVERAGE					
NAPHTALENE	<0.01	<0.01	<0.01	<0.01	<0.01
ACENAPHTHYLENE	<0.01	<0.01	<0.01	0.15	<0.01
ACENAPHTHENE	0.26	0.07	<0.01	0.14	<0.01
FLUORENE	<0.01	<0.01	<0.01	<0.01	<0.01
PHENANTHRENE	0.19	<0.01	<0.01	0.80	<0.01
ANTHRACENE	0.21	<0.01	<0.01	<0.01	<0.01
FLUORANTHENE	0.64	0.08	<0.01	0.18	0.08
PYRENE	1.21	0.12	<0.01	0.21	0.20
BENZO(a)ANTHRACENE	0.38	<0.01	<0.01	0.19	0.15
CHRYSENE	0.28	<0.01	<0.01	0.18	0.18
BENZO(b)FLUORANTHENE	0.57	0.26	0.41	0.23	1.57
BENZO(k)FLUORANTHENE	1.55	1.41	0.34	2.48	<0.01
BENZO(a)PYRENE	0.36	<0.01	<0.01	2.07	0.26
INDENO(1,2,3-cd)PYRENE	0.65	<0.01	<0.01	<0.01	0.35
DIBENZO(a,h)ANTHRACENE	<0.01	0.21	<0.01	<0.01	0.22
BENZO(g,h,i)PERYLENE	0.17	<0.01	<0.01	0.25	0.43
MEAN LEVELS FROM COLECTED SITES	6.47	2.17	0.75	6.91	3.44
BENCENIC RINGS					
2	0.58	0.16	<0.01	0.14	<0.01
3	3.14	0.24	<0.01	0.66	0.18
4	7.17	3.25	0.3	1.32	2.95
5	2.66	0.48	<0.01	1.17	2.21

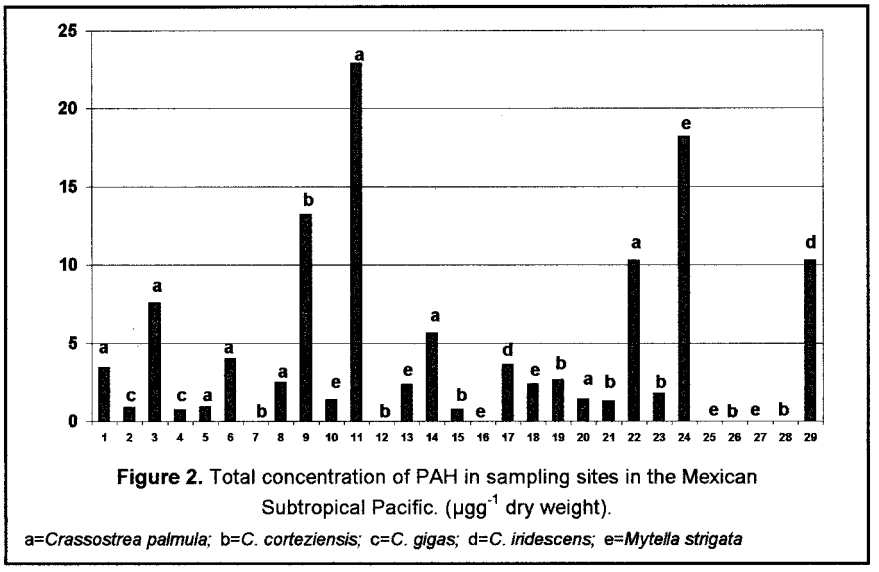

Figure 2. Total concentration of PAH in sampling sites in the Mexican Subtropical Pacific. ($\mu g g^{-1}$ dry weight).

a=*Crassostrea palmula;* b=*C. corteziensis;* c=*C. gigas;* d=*C. iridescens;* e=*Mytella strigata*

corteziensis (13.19μg/g) at 9 site in Ohuira, Sinaloa; *C. iridiscens* (10.24μg) at 29 site in Puerto Vallarta, Jalisco; the rest of the samples showed levels below 10μg/g.

C. iridescens is a marine specie associated to rocky substrates in sub-tidal areas. These specimens were collected in Puerto Vallarta (Jalisco) and in the Mazatlan Bay (Sinaloa), both ports have an intense marine traffic, large human settlements that include agroindustrial, fishery, and tourist activities, which are sources of PAH. On the other hand, *C. palmula* is a specie that lives associated to the roots of mangroves, where marine conditions predominate, in semi-enclosed water bodies of coastal lagoons and bays, whose hydrological conditions allow the residence time of PAH. The average level found in this specie (6.47μg/g) is mainly due to activities as maintenance and washing of vessels which are common in Pichilingue locality, La Paz, BCS. The mussel, *M. strigata*, inhabits the muddy plains, and is associated to mangrove roots and shallow muddy slimes of the coastal lagoons. For this specie, the greatest concentration belonged to the locality of Mexcaltitan, a region considered to be a lacunar complex located in the state of Nayarit, and which can function as a pollutants trap.

In the five analyzed species, the PAH benzo(b)fluoranthene predominates, the highest average concentration was found in *M. strigata* with 1.57 μg/g, followed by *C. palmula* with 0.57 μg/g, whereas in the other three species levels below 0.42 μg/g were recorded. Fluoranthene, pyrene, and benzo(k)fluoranthene were found in four of the five species. Chrysene, benzo(a)anthracene, benzo(a)pyrene and benzo(ghi)perylene were only found in *C. palmula*, *C. iridescens* and *M. strigata* (Table 1). It must be noted that in *C. gigas* only benzofluoranthenes were detected.

Considering the amount of benzene rings that constitute them, general distribution of PAH showed the following behavior: 4 > 5 > 3 > 2 (Table 1). This distribution suggests a mixed origin of the sources (pyrolysis and petrogenesis) from which the hydrocarbons are derived, i.e., coastal vegetation burning, industrial and urban drains and effluents from diverse types of vessels (Hong et al. 1995; Sherblom et al. 1995; Page et al. 1999). The PAH predominating in the five species of bivalve analyzed were as follows: benzo(k)fluoranthene (38%), benzo(b)fluoranthene (24%), pyrene (17%), benzo(a)pyrene (11%) and indeno(1,2,3,-cd)pyrene (10%).

Previous data reported in the Mussel Watch Program, indicated a range of concentrations for PAH of 0.1 to 0.6μg/g for oysters and mussels from the Pacific coast and the Gulf of Mexico (O´Connor, 1992; Lauenstein et al. 1995; Sericano et al. 1995; Cantillo et al. 1997). Indeed, Jaffé et al. (1995) reported mean concentrations of 0.04μg/g in the bivalve *Tivela mactroidea* from the Venezuelan littoral, where a high impact of oil activities is present.

Gold-Bouchot et al. (1997) established a range of concentrations of 0.01-0.24μg/g for *Crassostrea virginica*, in mexican areas of the Gulf of Mexico, and Jackson et al. (1994) reported a range of 0.21 to 0.35μg/g of PAH for the same

specie in the northern part of the Gulf of Mexico. In contrast, the concentrations of PAH determined in this study are higher than those found in previous published data, with values from 0.75 in *Crassostrea gigas* up to 6.9μg/g in *Crassostrea iridescens,* thus, indicating a high bioaccumulation process.

The presence and high concentrations of PAH in the organisms analyzed, indicate their bioavalability, and the fact to find a dominance of them with high molecular weights would indicate their fast sedimentation and later bioaccumulation by benthic species (Pereira et al. 1992; Baumard et al. 1998). In laboratory experiments, it has been observed that mollusks bivalves showed high bioconcentration to heavy PAH as benzo(a)anthracene with depuration rates of almost 30 days (D'Adamo et al. 1997).

The mollusks analyzed in this study are within the consumption habits of the Mexican market. Therefore a continuous surveillance as well as the establishment of the regulatory permissible limits for human consumption is indispensable not only in Mexico but throughout the world, since no international standards are available that limit the permissible concentration of these compounds in the marine organisms tissue.

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