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Analysis for Polycyclic Aromatic Hydrocarbons in Mussels (*Mytilus galloprovincialis*) from the Thermaikos Gulf, Greece

Helen G. Iosifidou, Stylianos D. Kilikidis, and Athanasios P. Kamarianos

Aristotelian University, Faculty of Veterinary Medicine, Department of Food Hygiene, Thessaloniki, Greece

Polycyclic aromatic hydrocarbons (PAH) are present in some petroleum products, coal tar, urban air particles, cigarette smoke, and gasoline and diesel engine exhausts (DUNN & FEE 1979). These compounds may occur naturally (e.g. crude oil), or be generated pyrolytically as a result of incomplete combustion in man-made or natural fires (NAS 1972). A number of PAH, including the widely studied compound benzo(a)pyrene, are potent carcinogens in animals and man.

PAH enter the marine environment from a variety of sources including petroleum pollution, industrial and domestic effluents, atmospheric particles, and biosynthesis by plants and microorganisms (DUNN & FEE 1979; HOWARD & FAZIO 1980). Several studies have shown the ability of marine organisms to accumulate petroleum hydrocarbons from amounts present in water (LEE et al. 1972; SUESS 1976). DUNN & STICH (1975) have shown that occurence of PAH in mussels may be a good indicator for the contamination of water with PAH. The present communication reports the results for the analysis for PAH in mussels (*M.galloprovincialis*) of the Thermaikos gulf of Greece (Fig. 1).

Fig. 1. Map of Greece.

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MATERIALS AND METHODS

The Thermaikos gulf area is suspected of being contaminated with petroleum hydrocarbons. A total of 57 samples were analyzed.The samples were taken as follows: 29 samples from the western area (industrial area) of the Thermaikos gulf and 28 samples from the eastern area (agricultural area) of the Thermaikos gulf (Fig. 2).

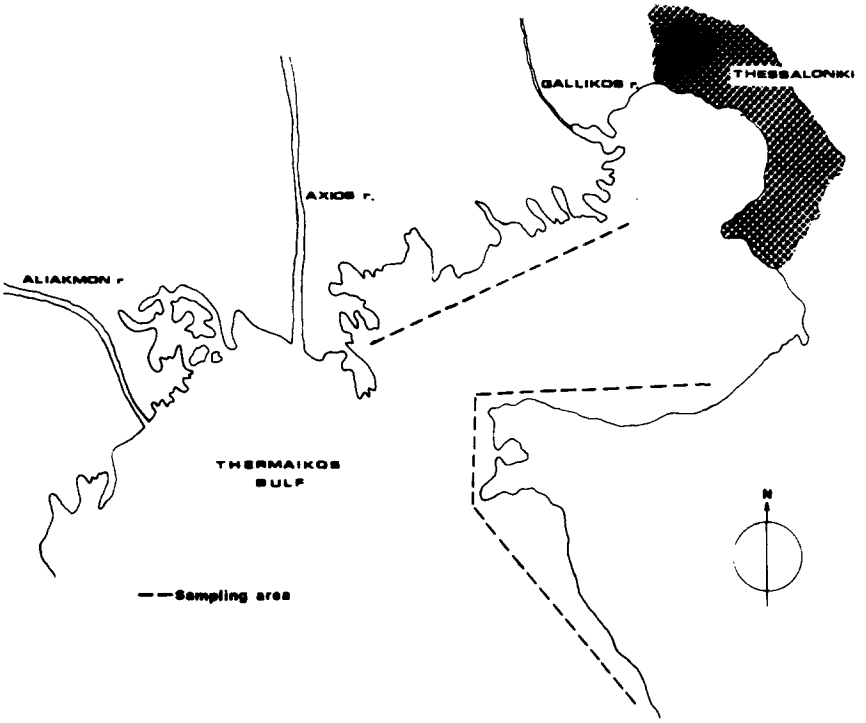

Fig. 2. Sampling areas of Thermaikos gulf.

For the extraction and purification of PAH from the marine samples, the method of HOWARD et al. (1966), as modified by the A.O.A.C. (1970) was used. Samples of 100 g (drained wet weight) of mussel tissue were refluxed with ethanol and KOH. Then, water was added and the PAH partitioned into isooctane. Column chromatography on Florisil into isooctane removed pigments. The PAH were eluted with benzene and concentrated into n-hexadecane. After that, the PAH were selectively extracted using dimethylsulfoxide (DMSO). The PAH were then recovered from the DMSO by the addition of water and back extraction into fresh isooctane. Isooctane extracts concentrated to 1 mL were analyzed for PAH using the chromatographic technique of KAMARIANOS (1980). According to KAMARIANOS technique, samples were analyzed in a gas chromatograph equipped with dual flame ionization detectors connected to parallel dual glass columns. Both columns were 200 cm X 6,35 mm X 2 mm i.d. and packed with 1.5% OV-17 coated on 80-100 mesh Supelcoport. The helium flow rate was 40 mL/min. Initial column temperature was 120 ^{o}C for 5 min; a program rate of 5 ^{o}C/min and a final temperature of 280 ^{o}C was used. The temperature of injection ports, as well as the temperature of detectors, was 300 ^{o}C. The air flow rate was 300

mL/min and the hydrogen flow rate was 30 mL/min. Before using, the columns were conditioned for 48 hours at 300 °C with a helium flow rate of 10 mL/min. Although the injectors septa were of low bleed, they also were conditioned for 48 hours at 300 °C with a helium carrier gas flow rate of 10 mL/min (Fig. 3).

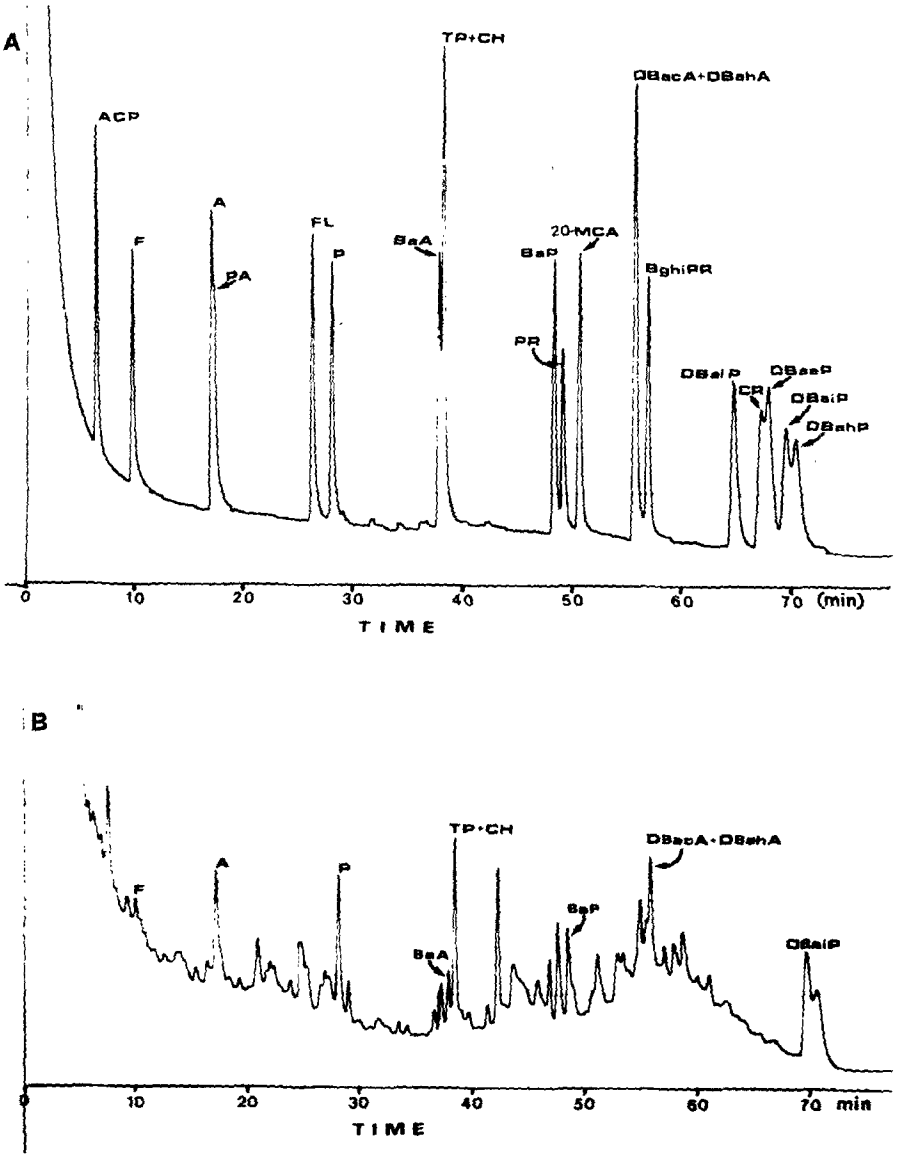

Fig. 3. A. Gas chromatogram of PAH standards. B. Gas chromatogram of mussels sample.

PAH were identified by comparing the retention times with those of standards (PAH) chromatographed separetely or added to the samples. The identification of PAH in the analyzed samples was also performed by comparing the retention times with those of standards using different chromatographic conditions (liquid phase, column temperatu-

re, and flow rate of carrier gas).

Periodically "blank" analyses were performed to check on contamination of the solvents. Control samples weren't performed because we didn't consider any area without contamination. The recovery of the method was 80-90%.

The identification of PAH would have been more accurate if Mass Spectrometry had been used.

RESULTS

Table 1 lists the levels of PAH found in samples taken from the western area of the gulf. The mussels contained 17 PAH, including: acenaphthene (ACP), fluorene (F), anthracene (A), phenanthrene (PA), fluoranthene (FL), pyrene (P), benzo(a)pyrene (BaP), perylene (PR), dibenzo(a,c)anthracene + dibenzo(a,h)anthracene (DBacA + DBahA), dibenzo(a,l)pyrene (DBalP), 20-methylcholanthrene (20-MCA), benzo(g,h,i)perylene (BghiPR), coronene (CR), dibenzo(a,e)pyrene (DBaeP) and dibenzo(a,i)pyrene (DBaiP). The levels found ranged from trace (less than 0.1 ng/g) to 18 ng/g (ppb).

Table 1. Concentration of PAH in mussels of the Thermaikos gulf (ppb wet weight).

Compound	Thermaikos gulf		
	Samples from the western area (n=29)	Samples from the eastern area (n=28)	Total samples (n=57)
ACP	7 (51.7)[a]	8 (67.9)	7 (59.6)
F	2 (10.3)	2 (14.3)	2 (12.3)
A	9 (55.2)	8 (53.6)	9 (54.4)
PA	9 (51.7)	9 (53.6)	9 (52.6)
FL	7 (51.7)	7 (60.7)	7 (56.1)
P	4 (55.2)	4 (53.6)	4 (54.4)
BaA	4 (41.4)	2 (25.0)	3 (33.3)
TP + CH	18 (69.0)	8 (35.7)	13 (52.6)
BaP	2 (20.7)	2[b] (3.6)	1 (12.3)
PR	14 (27.6)	1 (28.6)	8 (28.1)
20-MCA	12 (38.9)	13 (32.1)	12 (35.1)
DBahA + DBacA	2 (34.5)	1 (32.1)	2 (33.3)
BghiPR	5 (62.1)	4 (60.7)	4 (61.4)
DBalP	4[c] (6.9)	trace[b] (3.6)	trace (5.3)
CR	4 (31.0)	3 (32.1)	4 (31.6)
DBaeP	trace (13.8)	5 (14.3)	2 (14.0)
DBaiP	8[b] (3.4)	trace[b] (3.6)	4[c] (3.5)

[a] Positiveness per cent. [b] Single sample value.
[c] Average of two determinations.

Benzo(a)pyrene, a known carcinogen and the most widely studied PAH, was detected in 6 samples at concentrations of 1.5 ng/g (mean value). Two of the samples contained DBalP and only one sample contained DBaiP.

The total PAH in the mussels from the western area analyzed amounted to 110 ng/g and the carcinogenic PAH amounted to 38 ng/g.

The levels of PAH found in mussels (Table 1) of the eastern a-

rea of the gulf were somewhat unexpected, in view of the fact that this area is agricultural. The PAHs detected were the same with those mentioned above. The concentrations found ranged from trace to 13 ng/g (wet weight). Levels of some PAH including the noncarcinogenic ACP, PA, P, 20-MCA, and DBaeP were higher than those corresponding to the western area of the gulf. One out of 28 samples contained benzo(a)pyrene. Likewise, one of the samples examined contained trace of the carcinogenic PAH dibenzo(a,l)pyrene and dibenzo(a,i) pyrene (detection limit 0.1 ng/g).

In general, the mussels of the eastern area contained 77 ng/g (ppb) of total PAH and 14 ng/g of the carcinogenic PAH.

Mussels of the Thermaikos gulf were found contaminated with 17 PAH (Table 1), including the carcinogenic BaA, BaP, TP+CH, DBacA + DBahA, DBalP, and DBaiP.

It is noteworthy that all samples examined (n=57) were found contaminated. The levels detected ranged from trace to 13 ng/g (wet weight, drained). Seven of the samples examined contained BaP at an average level of 1 ng/g. DBaiP was found at trace levels in a small proportion of samples. Another compound, benzo(a)anthracene, was found in 19 samples at concentrations ranging from 2 ng/g to 42 ng/g. Dibenzo(a,h)pyrene was not found in any of the samples analyzed.

On the basis of the above data, the concentration of total PAH in mussels of the Thermaikos gulf amounted to 91 ng/g (wet weight) and those of the carcinogenic PAH counted to 19 ng/g.

DISCUSSION

The results show that the mussels of the Thermaikos gulf are contaminated with PAH. It should be pointed out that this contamination was not unexpected since, as reported by ALEXANDROU (1980) the contamination of the gulf with petroleum hydrocarbons has been occuring for a number of years. Furthermore, the results obtained indicate that there is a positive correlation between the levels of PAH and the carcinogenig PAH in mussels and industrial activity. Thus, the levels of contamination of the western area are higher than those of the eastern area. Considering the industrial activity of the area, these levels should be expected to be much higher. However, the present situation might be due to prevailing currents within the area of the gulf (Fig. 4).

From the samples analyzed, 17 PAH were isolated including 6 carcinogenic compounds. Their levels ranged from trace (less than 0.1 ng/g) to 13 ng/g (wet weight). Unfortunately, it is not possible to compare our findings concerning the concentrations of total PAH in mussels with those of other regions, owing to lack of sufficient data on levels of PAH in mussels, besides BaP. The concentrations of FL, P, PR and PA are substantially higher than those reported by FAZIO (1980) in the oysters of Galveston Bay.

The concentrations of BaP ranged from trace to 27 ng/g (average value 1 ng/g). These levels are lower than the reported levels of 540 mg/kg dry weight (SUESS 1970) 16-22 mg/kg dry weight (GREFFARD & MEURY 1967) and 55 µg/kg dry weight (MALLET et al. 1963) in mussels of the Italian, French and Greeland coasts, respectively. Consequently, the pollution of the Thermaikos gulf with BaP is low compared with the degree of pollution reported in the above areas. However, it should be pointed out that the levels of BaP only in mussels do

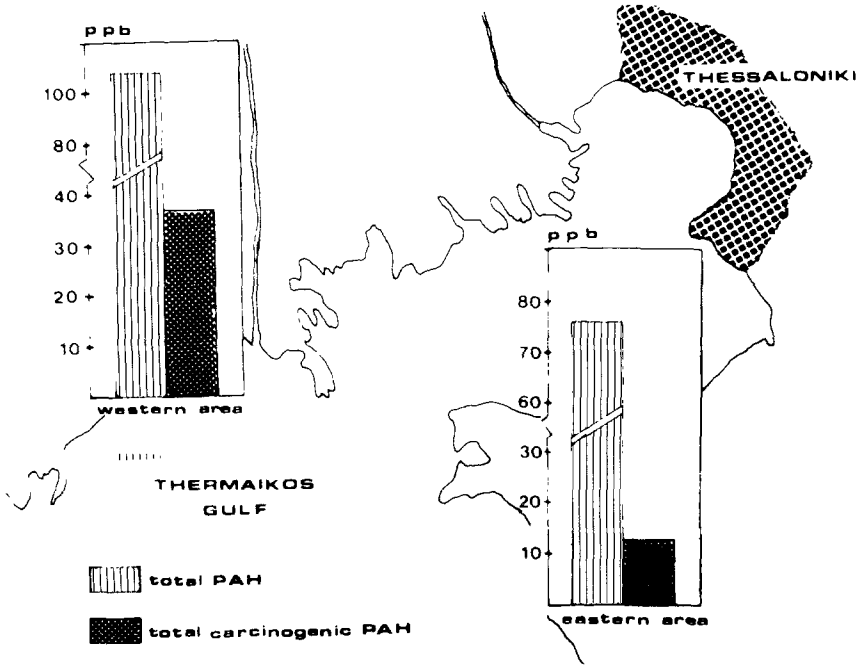

Fig. 4. PAH levels in mussels of western and eastern area of Thermaikos gulf.

not indicate the degree of pollution of an area, since it is possible for the mussels to be contaminated with other carcinogenic,e.g. PAH or PAH, of unknown toxicological significance.

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