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Polycyclic Aromatic Hydrocarbons in Mussel and Fish from the Finnish Archipelago Sea

Kristiina Rainio, Reino R. Linko, and Leena Ruotsila

Department of Chemistry and Biochemistry, Laboratory of Food Chemistry, University of Turku, SF-20500 Turku, Finland

Polycyclic aromatic hydrocarbons (PAH's) are widely distributed in the aquatic environment. Oil spills and incomplete combustion of fossil fuels are major sources of PAH's. Industrial and domestic effluents, terrestial contributions and biosynthesis by plants and micro-organisms are other sources.

Marine organisms are known to adsorb and accumulate PAH's from water. Mussels due to their widespread distribution in coastal waters have been studied in many laboratory and field experiments for their responses to PAH exposures (Ehrhardt and Heinemann 1975; Pancirov and Brown 1977; Kveseth et al. 1982; Iosifidou et al. 1982; Bjorseth et al. 1979;). The occurence of PAH's has been studied also in fish (Pancirov and Brown 1977; Neff et al. 1976; Humason and Gadbois 1982). This report presents preliminary data for the PAH content in blue mussel and fish from the Finnish Archipelago Sea.

MATERIALS AND METHODS

Mussel and fish samples were collected from the Finnish Archipelago Sea during 1978–1979 (Fig. 1). The sampling sites and times are given in connection with the results (Table 1 and 2).

Blue mussels (Mytilus edulis) were sampled in 1978 with an Agassiz-trawl, while in 1979 they were collected from algae (Fucus vesiculosus) by diving.

Baltic herring (Clupea harengus), which belongs to the migrating pelagic fish species and two bottom living fishes, pike-perch (Stizostedion lucioperca) and burbot (Lota lota) were netted by local fisherman.

The mussel and fish samples were wrapped in aluminium foil, frozen and stored at -20°C.

For analysis the mussels were partially thawed, the shells were removed and a composite sample of at least 10 individuals was made. Similar composite samples were obtained of Baltic herring

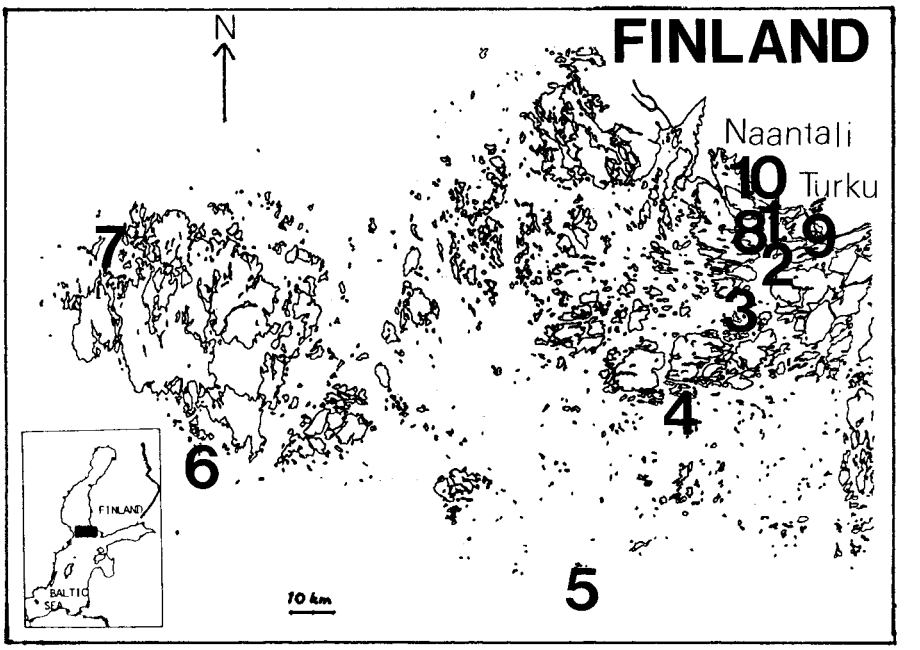

Figure 1. Sampling sites of mussel and fish in the Finnish Archipelago Sea.

after fileting and removing the skin. Pike-perch and burbot were analysed individually using samples of the back muscle, liver and gall prepared while still partially frozen.

The samples were homogenized with a macerator. Five gram sample of mussel homogenate was dried with anhydrous sodium sulphate and the hydrocarbons together with the fats were Soxhlet-extracted with a mixture of hexane, acetone, diethyl ether and petroleum ether (Bp 40-60°C) (2.5:7.5:1:9 v/v) for 6 hours. The solvents were evaporated to near dryness and the fatty residue was saponified by refluxing in aqueous methanolic (1:9 v/v) 2N KOH solution for 3 hours.

Samples of 90-120 g of fish muscle, 9 g of liver and 0.4-9.0 g of gall homogenate were digested by refluxing in 2N methanolic-KOH solution as described above.

Following the digestion, the methanol-KOH supernatant was extracted with cyclohexane according to the method of Grimmer and Böhnke (1975). To separate PAH's from the aliphatic hydrocarbons the liquid-liquid extraction procedure developed by Natusch and Tomkins (1978) was applied. The cyclohexane extract was partitioned three times with equal volumes of dimethylsulfoxide (DMSO). The DMSO layers, which contained the PAH's, were then combined.

For clean-up of PAH's two volumes of water were added to the combined DMSO extracts and the resulting solution was partitioned three times with equal volumes of cyclohexane. The cyclohexane layers were combined, washed once with water, dryed with anhydrous sodium sulphate concentrated to a volume of 50-100 µl and analysed by GLC and GLC-MS.

GLC-analyses were conducted on a Varian Aerograph, Model 3700 gas chromatograph equipped with a flame ionization detector. The glass capillary column 40 m long and 0.3 mm i.d. was coated with OV-1. The column temperature was programmed to rise at a rate of 6°/min from 120° to 280°C, after which the column was operated isothermally. The temperature of the injector block was 270°C and that of the detector 300°C. Nitrogen was used as the carrier gas at a flow rate of 1.5 ml/min.

High separation effiency was achieved in GLC-analysis. Thus a baseline separation was obtained for benzo(a)pyrene and benzo(e)pyrene. On the other hand benzo(a)anthracene, chrysene and triphenylene could not be resolved. The PAH's were identified tentatively by using relative retention indices and by coinjection with authentic standards. Identifications were confirmed by GLC-MS-analyses performed on a LKB-9000-apparatus with an ionization energy of 70 eV. The same OV-1 capillary column as above was used. The PAH's were quantified by comparing the peak areas with those of the standards. To check the purity of the solvents and reagents used in the extraction and clean-up procedure blank analyses were conducted periodically. No PAH-contamination was recognized by GLC.

In order to test the efficiency of the extraction and clean-up method for PAH's recovery experiments were performed with spiked samples. Naphthalene, phenanthrene, anthracene, fluoranthene, pyrene and triphenylene were added to the samples at the concentrations of 4-20 µg. The recoveries varied 49-97 %, the average recovery was 78 %.

RESULTS AND DISCUSSION

The results of the analyses of the PAH concentrations in mussel and fish collected during 1978-1979 from the Finnish Archipelago are shown in Table 1 and 2.

The mussel and fish samples were analyzed for naphthalene, biphenyl, acenaphthene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, triphenylene, benzo(e)pyrene, benzo(a)pyrene, perylene and benzo(ghi)perylene.

Of 14 PAH components studied naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)pyrene and compounds of benzo(a)anthracene/chrysene/triphenylene group were detected in mussel samples (Table 1). The concentrations of individual PAH components varied from < 0.5 (detection limit) to 109 µg/kg

Table 1. PAH concentration in mussel Mytilus edulis from the Finnish Archipelago (µg/kg wet weight). Sampling sites, see Fig. 1

Site No	Date	N[a]	Ph	A	Fl	Pyr	BaA Chr Tri	BaP	Total PAH
1	78-10-09	41	-	14	-	-	-	-	55
2	10-09	5	7	15	-	21	-	5	53
3	10-09	-	109	-	-	9	-	-	118
4	10-12	-	-	-	-	-	-	-	-
5	79-06-05	19	41	-	23	34	15	3	135
6	05-09	5	14	9	43	77	-	-	148
7	05-23	-	-	-	-	-	-	-	-

N[a] = naphthalene, Ph = phenanthrene, A = anthracene, Fl = fluoranthene, Pyr = pyrene, BaA = benzo(a)anthracene, Chr = chrysene, Tri = triphenylene, BaP = benzo(a)pyrene
- = concentration below the detection limit 0.5 µg/kg.

wet weight. However, they were very unevenly distributed among the different samples. Naphthalene, phenanthrene and pyrene were found in most of the samples, while benzo(a)pyrene in only two samples (sites 2 and 5). The concentrations of PAH's were slightly higher in mussels from the sampling sites 5 and 6 compared to other sites. These two areas were influenced by the oil spill of Antonio Gramsci in Baltic Sea during 1979 (Pfister 1980). On the other hand in two sampling sites (Nos 4 and 7) no PAH compounds could be detected.

Relatively few detailed studies have been made concerning the PAH levels in mussels. Moreover the comparison of the results obtained so far, is rendered difficult by the differences of analytical methodology (Howard and Fazio 1980). Also the type and number of the PAH components analyzed varies widely. This affects especially the total amounts of PAH's.

Also the natural background levels of PAH's in mussels are still largely unknown. However, Mackie et al. (1980) regarded areas, where the total PAH concentration in mussels varies within 50-140 µg/kg wet weight as unpolluted and those containing 1930-2850 µg of total PAH/kg wet weight as heavily polluted. Thus the total PAH levels, < 0.5 - 148 µg/kg, in mussels (Mytilus edulis) from the south-western coast of Finland correspond to that of unpolluted areas. In this respect they resemble mussels (species not reported) from Falmouth, Mass. USA (Pancirov and Brown 1977) and those (M. galloprovincialis) from Thermaicos Gulf, Greece (Iosifidou et al. 1982) with the total PAH content of 2.5-6.5 and 77-111 µg/kg wet weight, respectively.

On the other hand the total PAH's in mussels from Saudafjord, Norway, under the waste effluents of a ferro alloy smelter rose from 739 to 29946 µg/kg wet weight in Modiolus modiolus species and 2785 µg/kg wet weight in Mytilus edulis depending on the

distance from the source (Bjorseth et al. 1979). The dominant PAH compound in both mussels was benzo(a)fluoranthene. Elevated total PAH levels, from 534 to 1060 µg/kg wet weight, were also found in mussels collected from Oslo-fjord near the sewage treatment plant of Oslo (Kveseth et al. 1982).

The PAH contents in three fish species from the coastal waters of Turku and Naantali cities and further away in the Archipelago (cf. Fig. 1) are presented in Table 2. In addition to fish muscle, liver and gall tissues were studied in case of two bottom living species.

Table 2. Concentrations of PAH's in the muscle, liver and gall in fish from the Finnish Archipelago (µg/kg wet weight). Sampling sites, see Fig. 1

Species and site No	Date	Tissue	N[a]	Ph	Fl	Pyr	BaA Chr Tri	Total PAH
BALTING HERRING								
3	78-11-07	Muscle	-	-	-	-	-	-
8	10-20	Muscle	-	-	-	-	-	-
8	12-01	Muscle	-	-	27	6	-	33
PIKE-PERCH								
8	78-12-01	Muscle	-	8	4	3	15	30
9	79-07-03	Muscle	-	11	4	2	-	17
9	07-03	Liver	45	43	15	15	-	118
9	07-03	Gall	-	142	95	-	-	237
BURBOT								
10	79-04-28	Muscle	-	14	5	4	3	26
10	04-28	Liver	72	86	45	62	180	445
10	04-28	Gall	215	44	29	25	-	313

[a] abbreviations, see Table 1.
- = concentration below the detection limit 0.5 µg/kg.

Only few of the studied 14 PAH components were found in fish muscle. In only one of the three Baltic herring samples fluoranthene and pyrene were detected (Table 2). Howard and Fazio (1970) have reported earlier the same PAH compounds in herring in amounts of 1 µg/kg. In addition to these PAH components phenanthrene and compounds of benzo(a)anthracene/chrysene/triphenylene group were found in the muscle tissue of the bottom living pike-perch and burbot. The low level, < 0.5-27 µg/kg wet weight, of all these four PAH compounds is noteworthy as well as the absence of benzo(a)pyrene in the samples. However, benzo(a)pyrene have been reported in Greenland cod (Mallet et al. 1963), in sardine from Italian coast (Boucart and Mallet 1965) in cod, flounder and menhaden from the US Atlantic coast Long Island Sound and Raritan Bay,

New Jersey, (Pancirov and Brown 1977) in amounts varying from < 1.0 to 6.5 µg/kg wet weight.

The last mentioned three fishes contained furthermore < 0.3-2.0 µg/kg benzo(a)anthracene, pyrene and methylpyrene. Later Humason and Gadbois (1982) determined the total PAH concentrations for 14 components in the edible portions of winter flounder (Pseudopleuronectes americanus), windowpane (Scophtalmus aguosus) and red hake (Arophysate chuss) from the New York Bight area, Jew Jersey, getting values as high as 62-536 µg/kg wet weight. The largest PAH concentrations found were for phenanthrene. Concentrations of benzo(a)pyrene ranged from 2 to 22 µg/kg.

Although the bottom fishes from the Turku Archipelago contained low levels of PAH's in muscle tissue like the pelagic Baltic herring < 0.5-33 µg total PAH/kg wet weight (Table 2), elevated total PAH concentrations were found in the liver (118-445 µg/kg and in the gall (237-313 µg/kg) of pike-perch and burbot. In addition to the PAH compounds found in fish muscle tissues naphthalene was present in the liver of pike-perch and burbot and in the gall bladder of the latter in concentrations from 45 to 215 µg/kg. Fairly high concentrations of phenanthrene and fluoranthene were also detected in these organs. According to Neff et al. (1976) PAH compounds have a tendency to accumulate in the liver and gall bladder of fish. As in fish muscle tissues no benzo(a)pyrene was detected in the inner organs of pike-perch and burbot.

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