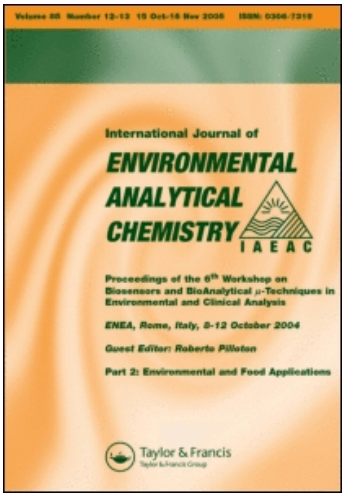

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Capillary Column Gas Chromatography of Environmental Polycyclic Aromatic Compounds†

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The methodologies are described for isolating clean fractions of polycyclic aromatic compounds from diverse environmental samples such as air particulate matter, sediments, and fish tissue. The common step in all procedures is the separation of the polycyclic aromatic compound fraction into well-defined chemical classes by adsorption chromatography on an alumina column. These procedures greatly facilitate the detailed characterization of the polycyclic aromatic hydrocarbons, sulfur heterocycles, and nitrogen heterocycles by capillary column gas chromatography.

KEY WORDS: Capillary column, gas chromatography, polycyclic aromatic compounds, air particulates, sediment, fish.

INTRODUCTION

It has long been known that chemical agents present in fossil fuels and produced during combustion of organic matter cause skin carcinomas in man. In addition, exposure of workers to vapors of synfuels (such as coal-derived liquids), coke-oven emissions, and coal processing emissions has been correlated with the increased incidence of cancers of other organ systems.[1] The polycyclic aromatic compounds (PAC) have been identified as major contributors to this activity. One sub-class of PAC, the

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polycyclic aromatic hydrocarbons (PAH), has been the most extensively studied in the past, but research to identify and assess the health effects of environmental PAC containing heteroatoms of nitrogen, oxygen, and sulfur is presently being pursued in many laboratories. These heterocyclic species have been shown in many cases to be the most significant environmental hazards. For example, the amino polycyclic aromatic hydrocarbons (APAH) have recently been shown to be the determinant mutagens in synthetic fuels,[2-4] and the nitro polycyclic aromatic hydrocarbons (NPAH) have been identified as direct-acting mutagens in carbon blacks[5] and diesel exhaust.[6,7] Several recent studies have also corroborated the observation that the polycyclic aromatic sulfur heterocycles (PASH) may be the most persistent of the PAC in the environment.[8-10] The importance of this observation is emphasized in light of recent tests [1,11] that show the PASH to be significantly more toxic than either the PAH or PANH (polycyclic aromatic nitrogen heterocycles).

The main pathways for human uptake of environmental PAC are ingestion, inhalation, or absorption through the skin. PAC are transported from the pollution source to man through the air and water. PAC can be inhaled or absorbed from air as a vapor, aerosol (such as in a coal conversion facility), or more commonly, as molecules adsorbed on particulate matter originating from an emission source. PAC enter the aqueous environment from a variety of sources including oil pollution, fallout from air pollution, effluents from industries and sewage treatment plants, and storm drain runoff. Man is most likely to encounter PAH in water by ingestion of contaminated food (such as fish) or drinking water.

In order to assess the effects of PAC on the environment and man, detailed characterization of PAC mixtures in various sample types must be done. Analysis of the distribution of these compounds in air particulates, water, sediments, and biological materials is important. The ideal analytical methodology would be one that could be uniformly applied to all sample types with comparable high quality results. Unfortunately, the vastly different sample matrices preclude the ideal situation, and modifications in procedures must be made for specific sample types. Nevertheless, it is still desirable to standardize as much of the analytical procedure as possible.

In this paper, the chemical class separation of PAC found in three different matrices (air particulate matter, sediment, and fish tissue) is described. The common aspects of the analytical methodology are the class separation of PAC by alumina column adsorption chromatography and the final separation of components by capillary column gas chromatography.

EXPERIMENTAL

Sample fractionation

Air particulate matter. A 15-g quantity of urban air particulate matter which was collected in the Washington, D.C., area over a 12-month period by the National Bureau of Standards, was extracted in a Soxhlet apparatus for 12 hrs with 250 ml of 1:3 methanol/benzene (v:v). The Soxhlet thimble and apparatus were pre-extracted with the same solvent mixture for 4 hrs before actual sample extraction.

The extract was concentrated to approximately 5 ml using a rotary evaporator, and a 1-ml portion was pre-adsorbed onto 3 g of neutral aluminum oxide (Brockman Activity 1, 80-200 mesh, Fisher No. A950), transferred to the top of a 6-g alumina column (10 cm × 11 mm i.d.), and eluted with 100 ml of chloroform containing 0.75% ethanol. The eluate was then reduced to a volume of 1 ml by rotary evaporation. This fraction was then transferred to a 22 mm i.d. column containing 60 g of Bio-Beads SX-12 (200–400 mesh, Bio-Rad), and eluted with methylene chloride. The first 55 ml which contained principally aliphatic compounds were discarded, and the next 100 ml which contained the PAC were collected and reduced in volume to 1 ml. It was found that this two-step alumina-gel column chromatographic procedure produced a relatively clean PAC fraction.

The chemical class fractionation of the PAC fraction was accomplished as described previously[13] for coal liquids on neutral aluminum oxide. Two fractions were collected for analysis in this work: the neutral PAC fraction containing PAH and PASH, and a more polar fraction containing mostly PANH. A schematic diagram of the fractionation procedure is given in Figure 1.

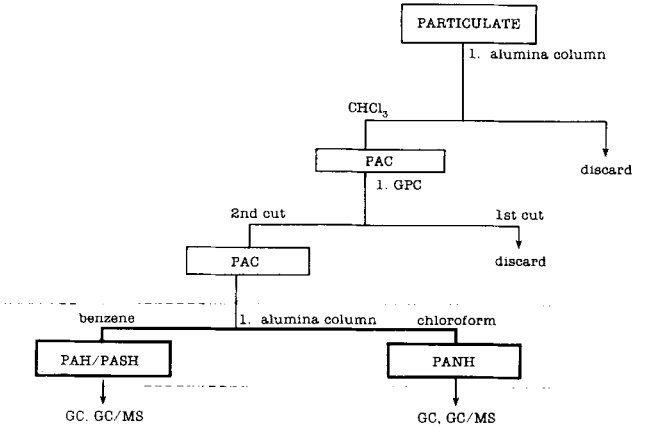

FIGURE 1 Fractionation scheme for air particulate matter.

Fish Tissue. Composited whole fish samples of a brown bullhead catfish (*Ictalurus nebulosus*) from the Black River in Ohio and a striped bass (*Morone saxatillis*) from the Sacramento River in California were provided by the U.S. Fisheries and Wildlife Service, Columbia National Research Laboratory, Columbia, MO. These samples were analyzed as previously described[14] and shown in Figure 2. Again, two fractions were collected from each sample for analysis: a PAH/PASH fraction and a PANH fraction.

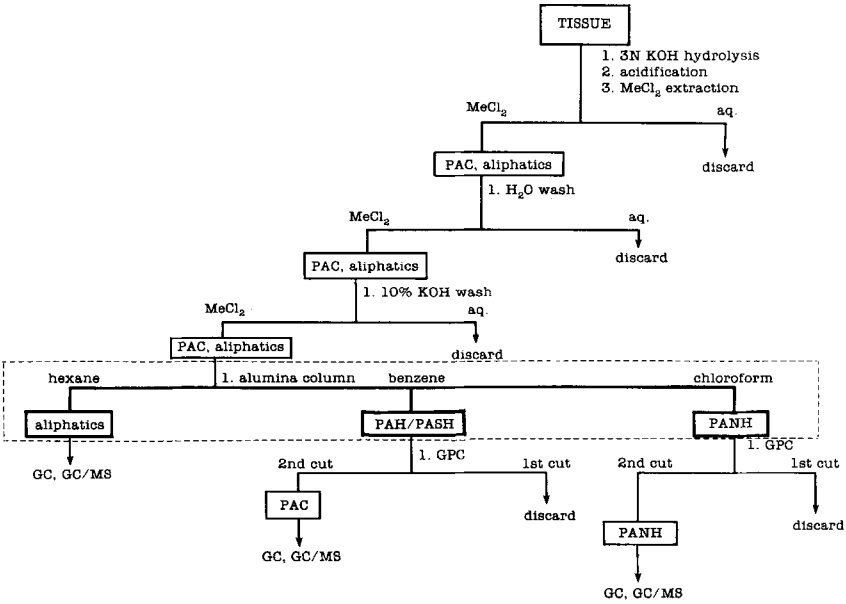

FIGURE 2 Fractionation scheme for fish tissue.

River Sediment. A river sediment sample from the Black River, also provided by the U.S. Fisheries and Wildlife Service, was extracted and fractionated according to the same scheme as shown in Figure 2, except the acidification step following the KOH hydrolysis and the acqueous washes following the methylene chloride extraction were omitted. Both PAH/PASH and PANH fractions were obtained.

Gas chromatography

Capillary column gas chromatography with flame ionization detection (FID) was performed using a Hewlett–Packard 5880 gas chromatograph with splitless vaporization injection. A flame photometric detector (FPD)

on a Perkin-Elmer Sigma III gas chromatograph was used to obtain chromatograms of the PASH. Fused silica capillary columns coated with SE-52 were prepared in this laboratory.

Mass spectral data of resolved components were obtained using a Hewlett–Packard 5982A quadrupole mass spectrometer which was operated in the electron impact mode at 70 eV electron energy. Spectra were acquired and processed with a Hewlett–Packard 5934A data system.

RESULTS AND DISCUSSION

As discussed earlier, no single analytical procedure is applicable to all sample types. In comparing Figures 1 and 2, it can be seen that the common step in the fractionation schemes is the alumina column separation of the PAC fraction into specific chemical classes. The virtues of this procedure have been discussed in detail elsewhere and applied to the characterization of PAC in coal liquids.[13] The main advantage is that clean, well-defined chemical classes of PAC are separated from each other for further chemical analysis. The various other steps in each fractionation scheme are designed to solve particular separation problems characteristic of the sample matrix analyzed. For instance, the two-step alumina and gel column clean-up was found essential to remove polar organic materials from the air particulate extracts before clean class separations could be obtained from the second alumina column. In contrast to this, the base hydrolysis, aqueous wash, and gel permeation steps were essential for separating the PAC from lipids and oils which were present in large amounts in the fish tissue.[14] When analyzing samples that are composed almost entirely of PAC, such as coal liquids, no fractionation steps are required prior to the alumina column class separation.[13]

Figures 3–5 show representative chromatograms of the PAH/PASH fractions isolated from air particulates, sediment, and fish tissue, respectively. All three fractions are almost entirely free of non-PAC compounds. This is essential for identifying PAC by retention measurements alone,[15] comparing chromatographic profiles, or facilitating identification by gas chromatography-mass spectrometry. Compounds identified in these fractions are listed in Table I. It is interesting to note the relatively high concentrations of PASH and alkylated PAC in the Black River fish. The ratio of phenanthrene concentration to dibenzothiophene concentration in the fish was 9:1 as compared to a ratio of 19:1 in the sediment (a sample of which was taken in the same vicinity as where the fish was caught). These results support other reports of selective bioconcentration of PASH in aquatic organisms.[8–10]

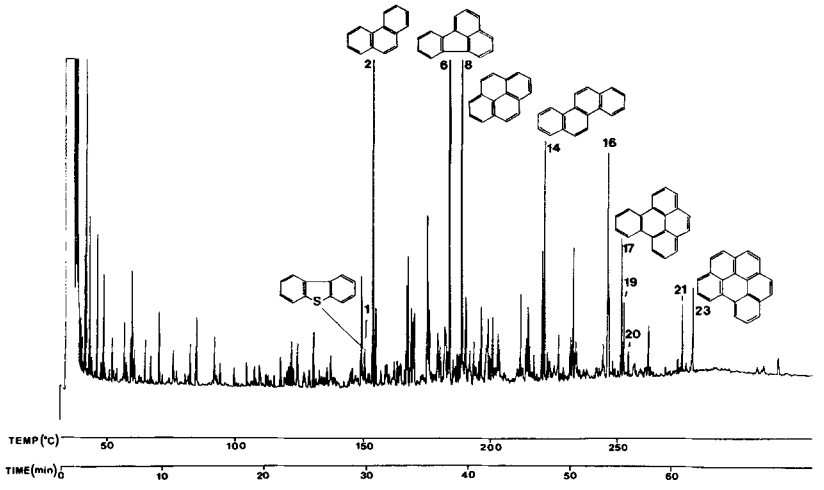

FIGURE 3 Capillary column gas chromatogram (FID) of PAH/PASH fraction from Washington air particulate matter. Conditions: 25 m × 0.20 mm i.d. fused silica capillary column coated with SE-52 (0.17 μm film thickness); oven temperature held at 40°C for 2 min, and then programmed at 4°C/min to 260°C; hydrogen carrier gas.

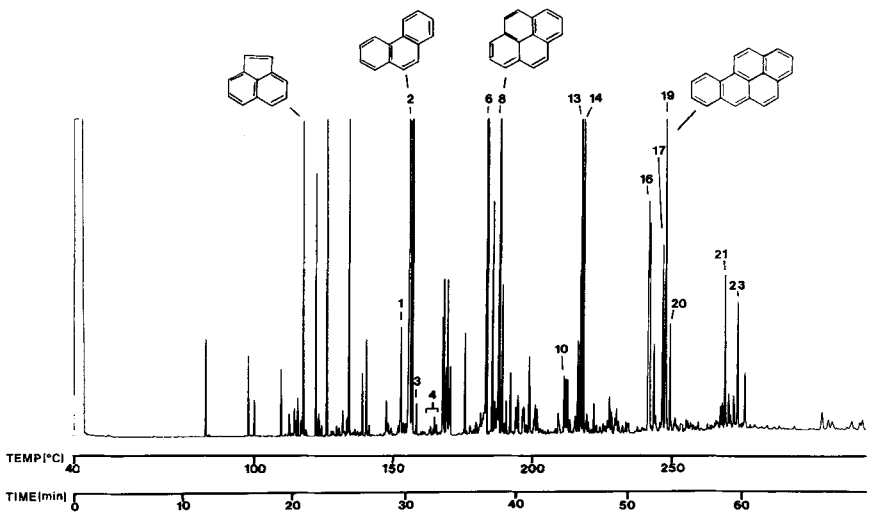

FIGURE 4 Capillary column gas chromatogram (FID) of PAH/PASH fraction from a Black River sediment. Conditions: 25 m × 0.20 mm i.d. fused silica capillary column coated with SE-52 (0.17 μm film thickness); oven temperature held at 40°C for 2 min, and then programmed at 4°C/min to 260°C; hydrogen carrier gas.

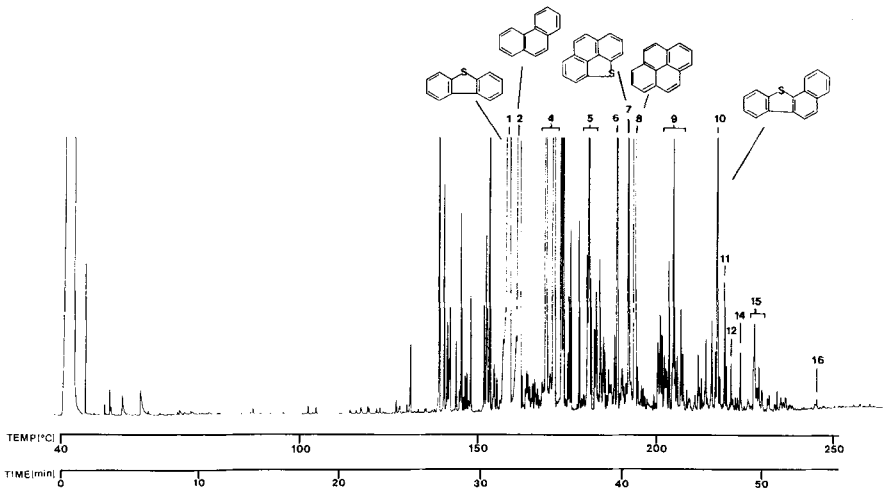

FIGURE 5 Capillary column gas chromatogram (FID) of PAH/PASH fraction from Black River bullhead catfish. Conditions: 20 m × 0.30 mm i.d. fused silica capillary column coated with SE-52 (0.34 μm film thickness); oven temperature held at 40°C for 2 min, and then programmed at 4°C/min to 260°C; hydrogen carrier gas.

Sulfur-selective chromatographic detection of the PASH in the neutral PAC fractions provides clearer comparisons of the distribution of sulfur-containing compounds in these fractions (Figures 6–8). A rather high relative concentration of alkylated PASH can be observed in the Sacramento River fish.

Representative chromatograms of the PANH fractions from air particulates, sediment, and fish tissue are shown in Figures 9–11. Compounds identified are listed in Table II. In all three samples, various carbazoles and azaarenes have been identified. It is also interesting to note the presence of several PAC containing keto-functional groups in the sediment and fish samples. The isoquinolines appear to be the major PANH components in the air particulate extract.

The efficient and reproducible methods described here for analyzing PAC in various sample matrices are important for proper utilization of high resolution gas chromatographic techniques. This is ultimately important for studies leading to the further understanding of the possible human health effects of environmental PAC.

TABLE I

Compounds identified in the PAH/PASH fractions of Washington air particulate, Black River Sediment, and Sacramento and Black River fish.

Peak #[a]	Compound Name
1	Dibenzothiophene
2	Phenanthrene
3	Naphtho[2,3-*b*]thiophene
4	Methyldibenzothiophenes
5	C_2-Dibenzothiophenes
6	Fluoranthene
7	Phenanthro[4,5-*bcd*]thiophene
8	Pyrene
9	Methylphenanthro[4,5-*bcd*]thiophenes
10	Benzo[*b*]naphtho[2,1-*d*]thiophene
11	Benzo[*b*]naphtho[1,2-*d*]thiophene
12	Benzo[*b*]naphtho[2,3-*d*]thiophene
13	Benz[*a*]anthracene
14	Chrysene/Triphenylene
15	Methyl 4-ring thiophenes
16	Benzo[*b*] & [*k*]fluoranthenes
17	Benzo[*e*]pyrene
18	Peri-condensed 5-ring thiophenes
19	Benzo[*a*]pyrene
20	Perylene
21	Indeno[1,2,3-*cd*]pyrene
22	cata-condensed 5-ring thiophenes
23	Benzo[*ghi*]perylene

[a]Peak numbers refer to peaks labelled in Figures 3–8.

TABLE II

Compounds identified in the PANH fractions of Washington air particulate, Black River Sediment, and Sacramento and Black River fish.

Peak #[a]	Compound Name
1	9H-Fluoren-9-one
2	Acenaphthoquinone
3	Benzo[*h*]quinoline
4	Acridine
5	Phenanthridine (Int. Std.) and Benzo[*f*]quinoline
6	Carbazole
7	Methylbenzoquinolines
8	Methylcarbazoles
9	Anthraquinone
10	Azafluoranthenes and Azapyrenes
11	Benzo[*def*]carbazole
12	Naphthoquinolines
13	Benzo[*a*]carbazole
14	7H-benz[*de*]anthracen-7-one
15	Benzocarbazole

[a]Peak numbers refer to peaks labelled in Figures 9–11.

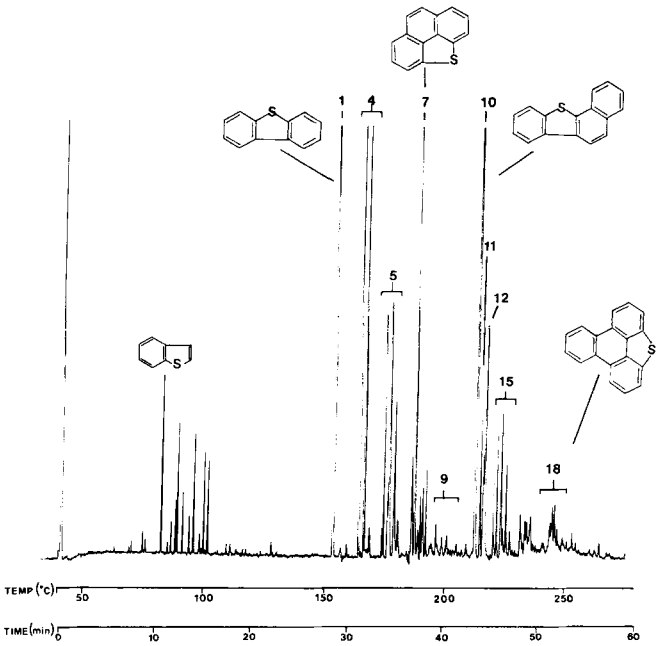

FIGURE 6 Capillary column gas chromatogram (FPD) of PAH/PASH fraction from Washington air particulate matter. Conditions: 25 m × 0.20 mm i.d. fused silica capillary column coated with SE-52 (0.17 μm film thickness); oven temperature held at 40°C for 2 min, and then programmed at 4°C/min to 260°C; helium carrier gas.

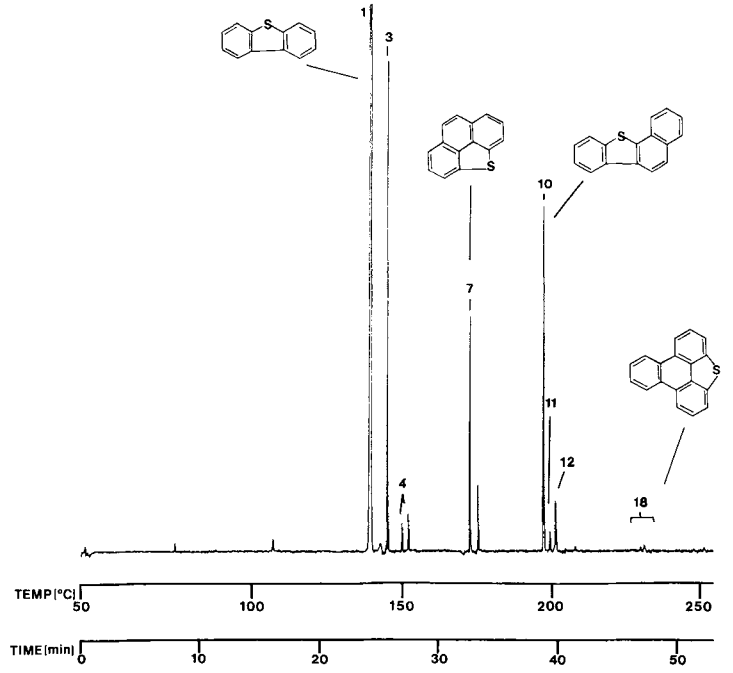

FIGURE 7 Capillary column gas chromatogram (FPD) of PAH/PASH fraction from a Black River sediment. Conditions: 20 m × 0.30 mm i.d. fused silica capillary column coated with SE-52 (0.34 μm film thickness); oven temperature held at 50°C for 2 min, and then programmed at 4°C/min to 265°C; helium carrier gas.

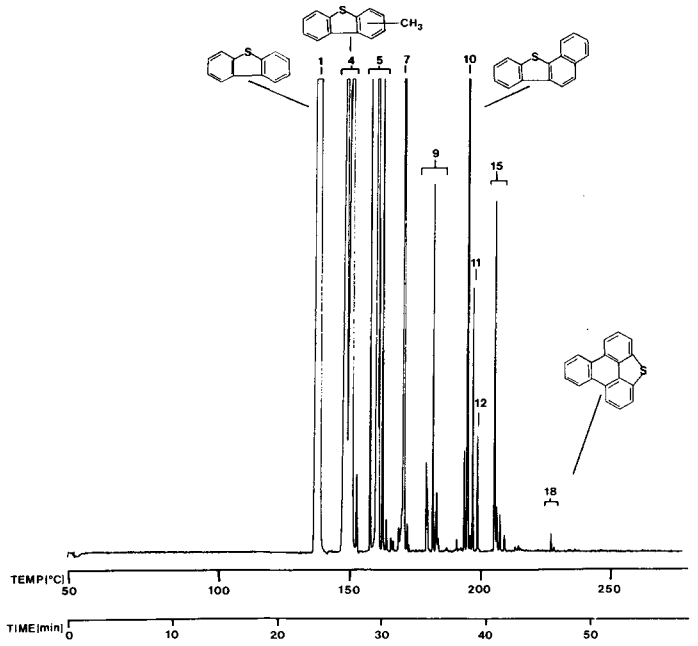

FIGURE 8 Capillary column gas chromatogram (FPD) of PAH/PASH fraction from a Sacramento River striped bass. Conditions: 20 m × 0.30 mm i.d. fused silica capillary column coated with SE-52 (0.34 μm film thickness); oven temperature held at 50°C for 2 min, and then programmed at 4°C/min to 265°C; helium carrier gas.

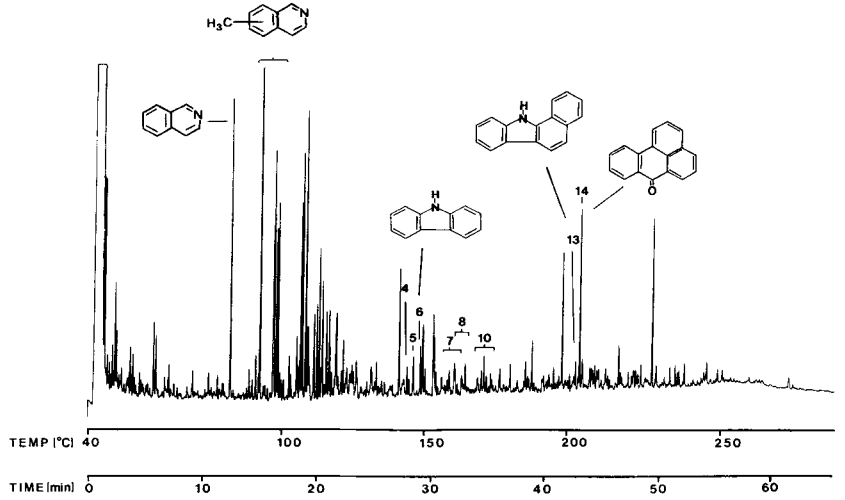

FIGURE 9 Capillary column gas chromatogram (FID) of PANH fraction from Washington air particulate matter. Conditions: 25 m × 0.20 mm i.d. fused silica capillary column coated with SE-52 (0.17 μm film thickness); oven temperature held at 40°C for 2 min, and then programmed at 4°C/min to 260°C; hydrogen carrier gas.

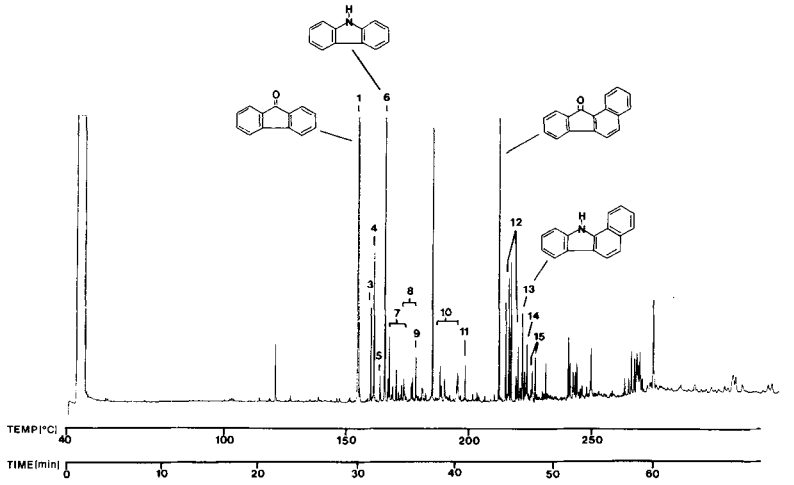

FIGURE 10 Capillary column gas chromatogram (FID) of PANH fraction from Black River sediment. Conditions: 25 m × 0.20 mm i.d. fused silica capillary column coated with SE-52 (0.17 μm film thickness); oven temperature held at 40°C for 2 min, and then programmed at 4°C/min to 260°C; hydrogen carrier gas.

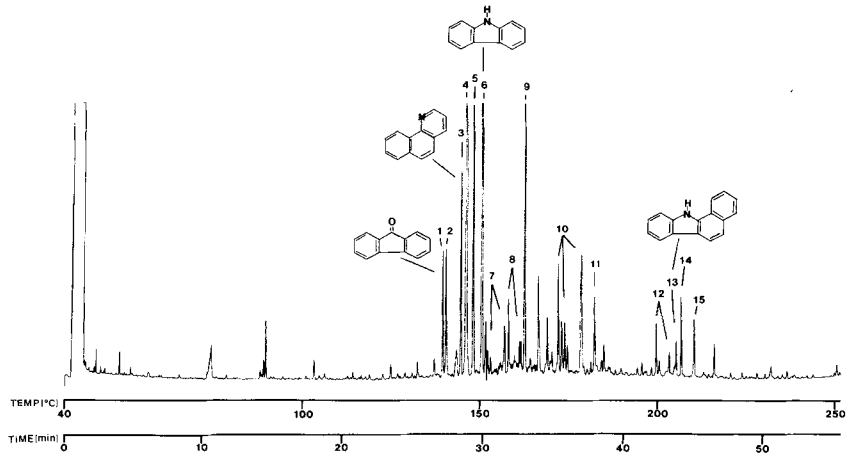

FIGURE 11 Capillary column gas chromatogram (FID) of PANH fraction from Black River bullhead catfish. Conditions: 25 m × 0.20 mm i.d. fused silica capillary column coated with SE-52 (0.17 μm film thickness); oven temperature held at 40°C for 2 min, and then programmed at 4°C/min to 260°C; hydrogen carrier gas.

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