A screen for Benzo(a)pyrene in Fish Samples From Crude Oil Polluted Environments

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Abstract: Several studies have shown that exposure to Benzo(a)pyrene increases the risk of cancer. In this study several fish samples from Niger the Delta region of Nigeria were screened for the presence of Benzo(a)pyrene. The study was carried out using Gas chromatograph coupled to a mass spectrometry detector. Benzo(a)pyrenes in the samples were identified through both retention time match with authentic standards and simultaneous maximization of several major ions from GC/MS data. Perylene-$_{d12}$ was used as the internal standard for quantitation. Concentration of benzo(a)pyrene in the samples ranged from 1.47 to 10.53 µg/kg which is more than WHO recommended maximum allowable concentration. Therefore this study concludes that the population is at an elevated risk of cancer of occurrence.

Keywords: cancer, delta region of Nigeria, chromatograph

INTRODUCTION

Benzo(a)pyrene (BaP) (Fig. 1) is a five-ring polynuclear aromatic hydrocarbon (PAH), belonging to the alternant class of PAHs [1]. It is known to be a ubiquitous environmental carcinogen. BaP has evoked much interest due to its carcinogenic properties. A variety of tests have shown BaP to be genetoxic. It has produced stomach tumor, mammary gland tumor, lung and respiratory tumors, hepatic tumors in laboratory animals[2]. Human exposure to BaP occurs through several routes namely the skin, the gastrointestinal tract and the pulmonary tract[3]. It is generated by incomplete combustion of organic materials arising in part from natural combustion such as forest fires and volcanic eruption[4 - 6]. Anthropogenic sources such as industrial production, transportation and waste incineration generate significant amount of this compound and other PAHs[7]. Petroleum production, import and export of petroleum product also contribute a lot to the extent of BaP contamination especially in marine samples. Other sources include crude oil contamination of ground water and fishes, tobacco smoke, exhaust fumes and combustion of all forms of fossil fuel.

The carcinogenesis of BaP is basically a result of its ability to bind to the DNA thereby causing a series of disruptive effects that end up in tumor initiation. The aromatic hydrocarbon receptor plays a very important role in this. Benzo(a)pyrene induced carcinogenesis involves 3 stages. First is the enzymatic activation of the compound into metabolites [8]. The second stage in the carcinogenesis of benzo(a)pyrene is the covalent bonding of anti-BPDE to DNA to form BPDE-DNA adduct [9]. The last stage is the induction of mutations that serve to initiate the transformation process as a result of BPDE-DNA adducts [10].

PAHs never occur as individual compounds in the environment [11], they occur as a mixture of many other polynuclear aromatic hydrocarbons. Several findings have revealed that weakly or non carcinogenic PAHs present in a mixture can modify the carcinogenic activities of a given polynuclear aromatic hydrocarbons such as Benzo(a)pyrene in rodent[12 - 15].

Benzo(a)pyrene being fat soluble, tend to bioaccumulate in marine organisms[16] in contaminated coastal environments, the Niger Delta being one of such environments. The Niger Delta region contains both fresh inland waters and a highly urbanized brackish ecosystem impacted by municipal and industrial activities, and more significantly by the extensive petroleum production activities in the area. A major concern is that fish from this region represent a significant component of the diet of a large percentage of the population, and this could present a number of

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human health problems. In this study, seventeen fish samples from four different species, namely Parachanna Obscura, Pseudolithus Elongatus, Lizza Dumerillii and Clarais Gariepinnus, were screened for the presence benzo(a)pyrene using GC/MS. Parachanna Obscura and Clarais Gariepinnus are fresh water fishes while Pseudolithus Elongatus and Lizza Dumerillii are from brackish ecosystem. This study targets these four species of fish because of their wide spread consumption in the Niger Delta and environs. The study revealed a relatively high degree of benzo(a)pyrene accumulation in all the fish samples.
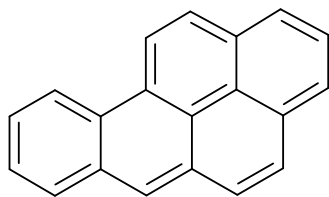
Fig 1: Chemical Structure of Benzo(a)pyrene.

EXPERINMENTAL

Collection of samples: The fish samples were collected directly from their natural ecosystem. Two species Pseudolithus Elongatus and Lizza Dumerillii were sampled from brackish ecosystem, while Clarais Gariepinnus and Parachanna Obscura were sampled from fresh water ecosystem. The Nigerian Institute of Oceanography and Marine Research Lagos identified the fish species.

Reagents: All chemicals and reagents were of analytical grade and of highest purity possible. LC-grade dichloromethane used for extractions was obtained from Fischer Scientific, Silica gel used to clean up the extracts was supplied by BDH Laboratories. A PAH standard mixture (NIST, Baltimore, MD) containing naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]flouranthene, benzo[k]flouranthene, benzo[a]pyrene, benzi[ghi]perylene dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene was used in this study. A mixture containing four isotopically labeled PAHs (ChemService, Westchester, PA) namely acenaphthalene-$_{d10}$, chrysene-$_{d12}$, phenanthrene-$_{d10}$ and perylene-$_{d12}$ was used as an internal standard.

Soxhlet Extraction: 10g of fresh fish fillet was homogenized in a mortar with about 10g of Na_2SO_4[17]

until a completely dry homogenate was obtained. The Soxhlet apparatus consisted of a 250 mL round bottom flask, condenser and extractor tube, seated in a temperature-controlled heating mantle. A Fischer brand rotovap was used for evaporating the extracts to the desired concentration. The homogenate was carefully transferred into the extraction thimble and placed in the extraction chamber of a Soxhlet extraction unit. Soxhlet extractions were carried out using a modified form of the EPA 3540 method[18] using 150 mL dichloromethane for 16 hours. The extract was concentrated in a rotavap.

Post-Extraction Cleanup: The extract was concentrated to 1 mL and loaded onto a silica gel column. The silica gel column was prepared by loading an activated silica gel onto a chromatographic column (1 cm internal diameter) to about 5 cm. An additional 1 cm of anhydrous Na_2SO_4 was added to the column. This was conditioned with methylene chloride. 1 mL of concentrated extract was loaded and eluted with 10 mL of methylene chloride[19]. This procedure was able to remove the very polar lipids off the extract. The lipids, being very polar, were adsorbed at the top of the column. Prior to analysis 0.5 µg of four internal standards were added to each of the sample extract and the volume reduced to 1 mL.

Analysis: The GC/MS system used for the analyses consisted of a Finnigan Magnum instrument equipped with a CTC A200S autosampler, a 30 m, 0.25 ID DB-5ms fused silica capillary column (J & W Scientific, Folson CA), and the Finnigan Magnum data system. Helium was used as the carrier gas and the column head pressure was maintained at 10 psi to give an approximate flow rate of 1 mL/min. The injector and transfer line were maintained at $290^{o}C$ and 250 C respectively. All injection volumes were 1 µL in the splitless mode. The column temperature was initially held at $70^{o}C$ for 4 minutes, ramped to $300^{o}C$ at a rate of $10^{o}C$/min, then held at $300^{o}C$ for 10 minutes [20]. The mass spectrometer was used in electron ionization mode and all spectra were acquired using a mass range of m/z 50 – 400 and automatic gain control (AGC).

RESULTS AND DISCUSSION

Chromatographic study: GC conditions were set using conditions similar to those in related studies [20]. Even though the target compound is BaP, in this study GC conditions and standards for sixteen priority PAHs (which BaP is part of) are employed. The column

temperature program gave baseline separation of the target compounds in a reasonable amount of time, with BaP eluting after 27.18 minutes (Fig. 2).
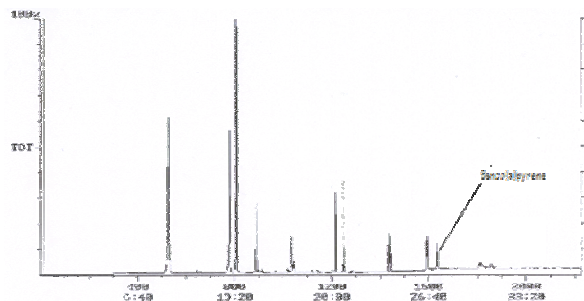

Fig. 2: Total ion chromatogram for the standard used in the study. BaP is highlighted. The chromatographic conditions are as described above. The retention time of BaP is 27.18 mins.

To evaluate the extraction efficiency of BaP from fish matrix, recovery studies were carried out using Perylene-$_{d12}$ (Fig. 3). Both BaP and Perylene-$_{d12}$ posses five aromatic rings and hence perylene-$_{d12}$ is a good surrogate for BaP. The percent recovery of 118% was obtained, and this demonstrates no significant losses of analytes during the sample workup process. The surrogate percent recovery was calculated using the equation:

$$\%R \;=\; \frac{Q_d}{Q_a} \;\times\; 100$$

Where Q_d is the quantity determined by analysis; and Q_a is the quantity added. For surrogate percent recovery to be acceptable it must fall between 60 and 120%[21]
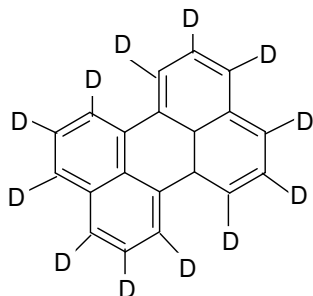

Fig. 3: Chemical structure of Perylene-$_{d12}$

Several dilutions of the standard were analyzed to determine the limit of detection (LOD), limit of quantitation (LOQ), limit of linearity (LOL), relative standard deviation (RSD) and regression coefficient (r^2). The LOD was determined by the signal to noise ratio of 3:1. The LOQ was determined by the signal to noise ratio of 10:1. The LOL was determined from the plot of the concentration versus response. The RSD was determined by triplicate of each analysis. The r^2 was determined for the compound using Microsoft Excel formula software. Fig. 4 shows the calibration curve for BaP obtained using a series of varying concentrations of BaP. The curve is linear with correlation coefficients from the linear regression of 0.995. Relative standard deviation from replicate analyses of the same standard (n = 3) was 2.11% (Table 1). Limits of detection and quantitation (LODs and LOQs) were 0.15 µg/mL and 0.5µg/mL respectively. BaP in the samples was identified by a combination of a retention time match and mass spectral match against the calibration standards. Quantitation was performed by the method of internal standardization using Perylene-$_{d12}$ as the internal standard based on the ratio of the peak height of the BaP ion to that of the internal standard. Fig. 5 shows the total ion chromatogram of a representative fish extract analyzed in this study.

Table 1: Analytical Characteristics of the compound

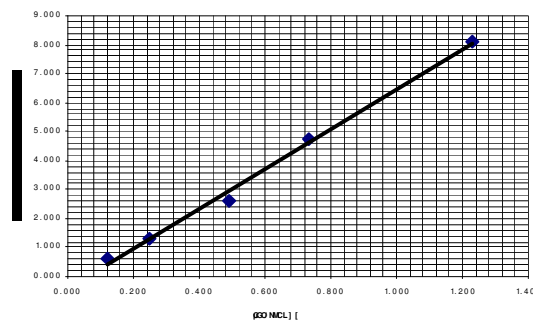| Compound name	Benzo(a)pyrene
Retention time	27.18 minutes
Linearity range	0.123 – 1.228 µg/ml
Regression coefficient	0.995
Relative Standard Deviation	2.11%
Limit of Detection	0.15 µg/ml
Limit of Quantitation	0.50 µg/ml
Important m/z	252, 225, 161, 126, 74
Molecular Formular	$C_{20}H_{12}$
Molecular weight	252

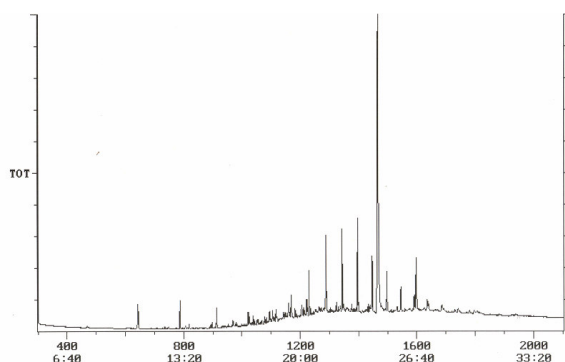Fig. 4: Calibration curve for BaP

Fig. 5: Total ion chromatogram of a representative fish extract analyzed in this study. Chromatographic conditions are as described above
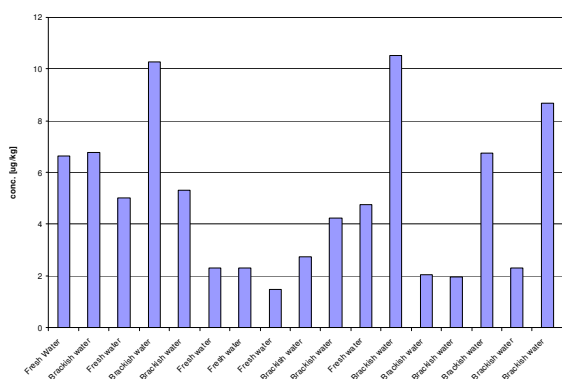

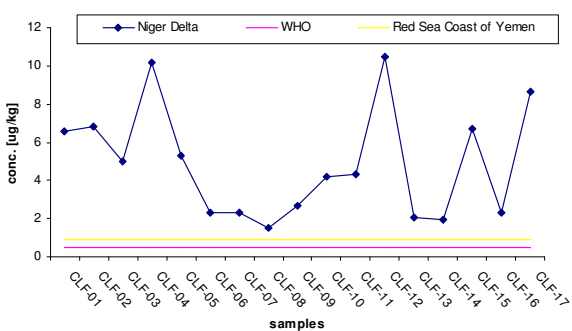Fig. 6: BaP distribution in fish of different ecosystems

Fig. 7: BaP profile in the studied fish samples compared with WHO benchmark and results Red Sea Coast of Yemen.

Benzo(a)pyrene Concentration in the Samples: All 17 samples contain BaP in significant amount indicating a high level of contamination. The concentration levels ranged from 1.5 µg/kg to 10.2 µg/kg. Table 2 shows the concentration levels of BaP in the studied samples. The BaP concentration did not show any particular trend in different species of fish. Both fresh water and brackish water species have concentration close to each other with the exception of a few deviation (Fig. 6). Therefore we can say that the level of BaP accumulation is a function of the level of contamination in a given environment. Fig. 6 illustrates this better.

Table 2: BaP concentrations in different fish samples

Fish sample	Type of Sample	BaP conc. in ug/kg
Sample 1	Fresh Water	6.64
Sample 2	Brackish water	6.78
Sample 3	Fresh water	5.01
Sample 4	Brackish water	10.27
Sample 5	Brackish water	5.32
Sample 6	Fresh water	2.32
Sample 7	Fresh water	2.3
Sample 8	Fresh water	1.47
Sample 9	Brackish water	2.74
Sample 10	Brackish water	4.24
Sample 11	Fresh water	4.77
Sample 12	Brackish water	10.53
Sample 13	Brackish water	2.06
Sample 14	Brackish water	1.98
Sample 15	Brackish water	6.74
Sample 16	Brackish water	2.3
Sample 17	Brackish water	8.67

Fig. 7 shows the profile of benzo(a)pyrene in the studied fish samples. The profile is compared with the WHO recommended background concentration of benzo(a)pyrene of 0.5 µg/kg in food samples [22] and the result obtained from Red Sea Coast of Yemen [23]. The Fig. clearly shows that the studied samples contain BaP in concentrations higher than the WHO recommended minimum and when compared with another environment in this case Red Sea coast of Yemen, with average concentration of 0.9 µg/kg, it shows that the level of contamination is much more in this environment too.

BaP like many other PAHs bioaccumulates in fish tissues because of their lipophilic nature [16]. Based on estimated fish consumption by the population of 40 g/day, the daily intake of carcinogenic BaP can be calculated as:

$$[40 \text{ g/person/day} \times \{C_{BaP}\}_a \text{ µg/kg}] / 1000 \text{ g/kg}$$

where C_{BaP} is the concentration of BaP in an individual fish sample studied. $\{C_{BaP}\}_a$ is the mean of BaP in the sample population. From table 2 the mean concentration of BaP for the samples is 4.95μg/kg, therefore the daily consumption of BaP can be given as:

[40 g/person/day x 4.95μg/kg] / 1000 g/kg = 0.198 μg/person/day

Fish is a major source of PAH exposure to man [24]. Its contribution to food is influenced by method of preparation [25]. Smoking and charboiling are additional sources of carcinogenic PAHs [26]. Other food sources such as vegetable, fruits and cereal from the area are bound to be contaminated, hence adding to the daily intake of carcinogenic PAHs by the population. This implies that this population is exposed to high level of this toxicant which has an adverse health implication.

CONCLUSION

The continued and long exposure of man to BaP may significantly influence the high rate of prostrate hyperplasia in men residing in these areas, as well as other toxicological and carcinogenic manifestations in humans.

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