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Full Length Research Paper

Assessment of polynuclear aromatic hydrocarbon content in four species of fish in the Niger Delta by gas chromatography/mass spectrometry

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Analysis for the presence of sixteen priority polynuclear aromatic hydrocarbons (PAHs) (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, dibenz[a, h]anthracene and indeno[1,2,3-cd]pyrene) was carried out on four different species of fish found in the Niger Delta region of Nigeria. The fish species included *Parachanna obscura*, *Pseudolithus elongatus*, *Liza dumerillii* and *Clarais gariepinnus*. Individual PAHs were identified through both retention time match with authentic standards and simultaneous maximization of several major ions from gas chromatography/mass spectrometry (GC/MS) data. Four isotopically-labeled internal standards namely D10-acenaphtalene, D12-chrysene, D10-phenanthrene and D12-perylene, were used for quantitation. All four species of fish were found to contain high levels of PAHs ranging from 0.41 to 39.64 ug/kg. The high molecular weight PAHs such as benzo[ghi]perylene, dibenzo[a,h]anthracene and indeno[1,2,3-cd]pyrene were consistently present in much higher amount than other PAHs in all four species of fish studied, suggesting higher resistance of these compounds to degradation.

Key words: PAHs, GC/MS, Fish, Niger Delta.

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are ubiquitous in marine environments and are often found in high concentrations around urban centers (Harvey, 1997). They are a major class of environmentally hazardous organic compounds due to their known or suspected carcinogenicity (Martson et al., 2001; Wynder and Hoffmann, 1959; Simko, 2002; Klein, 1963). Some of them are fat soluble and tend to bioaccumulate in living organisms, especially those higher up in the food chain (Atlas, 1991). Major sources of PAHs in the coastal environment include drilling operations, petroleum production, transportation activities, and combustion of fossil fuel (Nwachukwu, 2000; Baek et al., 1991; Yang et al., 1998; Lorber et al., 1994; Grova et al., 2002). PAH contamination in food and effect on human health have been of major concern (Lawrence and Weber, 1984; Alonge, 1988). Several studies have detected PAHs in mussels, snails and fish from contaminated waters (Sirota and Uthe, 1980; Speer et al., 1990; Uthe and Musial, 1988).

The Niger Delta region of Nigeria has had extensive petroleum production activities over the past few decades. This delta has been polluted by seepages from the oil discharge terminals as well as spillage of crude oil from production facilities. This has significantly contributed to pollution problems in this surrounding environment. One particular 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 human health problems. One goal of this study was to assess the types and levels of PAHs in several varieties of fish in the delta, and provide meaningful data to make appropriate recommendations on the long-term implications on human health.

Four species of fish, namely *Parahanna obscura, Pseudolithus elongatus, Lizza dumerillii* and *Clarais gariepinnus,* were studied for the presence of PAHs. *P. Obscura* and *C. gariepinnus* are fresh water fishes while *P. elon gatus* and *L.. dumerillii* were from brackish ecosystem. This study targets these four species of fish because of

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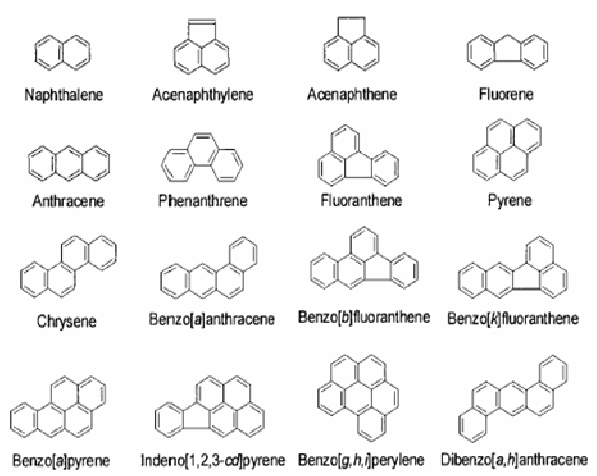

Figure 1. The chemical structure of the 16 target PAHs.

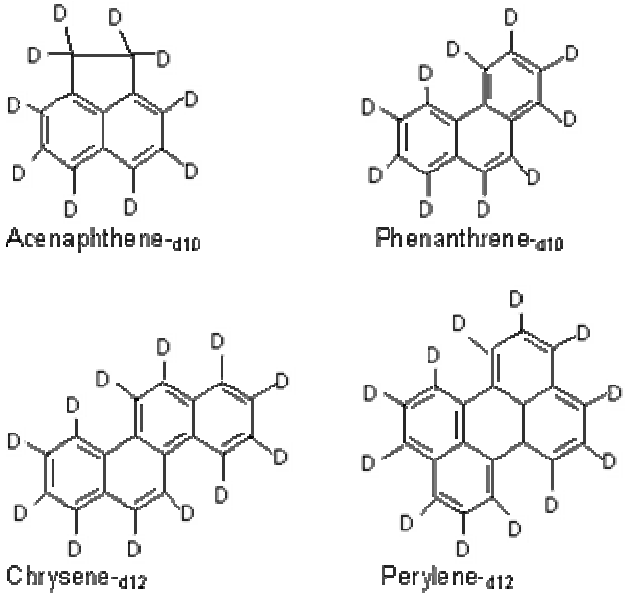

Figure 2. The chemical structures of the 4 isotopic PAHs used as internal standard.

their wide spread consumption in the Niger Delta and environs. The study revealed a high degree of PAH contamination in all four species.

MATERIAL AND METHOD

Study site

The Niger Delta is a wide expanse of swamp forest ecosystem extending from the Atlantic Ocean to the River Niger. It is located between longitude 5° and 9° E and between latitude 4° and 6° N. Along its southern side, Niger Delta is separated from the Atlantic Ocean by a band of Mangrove which can reach up to 10 km inland. In front of the mangrove belt and close to the sea are ephemeral coastal barrier islands often clothed in transitional vegetation. The ecoregion's total area of approximately 15,000 km^2 is contained in three states of Nigeria namely: Rivers, Bayelsa, and Delta. It contains both fresh inland waters and a highly urbanized brackish ecosystem impacted by municipal and industrial activities that have significantly increased in the past decades. It is environmentally impacted due to significant and large-scale activities of import and export of petroleum-related products. The choice of the location was influenced by the fact that it is one of the most significant environments in Nigeria.

Collection of samples

The fish samples were collected directly from their natural ecosystem. Two species *P. elongatus* and *L. dumerillii* were sampled from brackish ecosystem, while *C. gariepinnus* and *P. 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 and 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]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene was used in this study. The chemical structures of each of these target compounds are given in Figure 1. A mixture containing four isotopically labeled PAHs (ChemService, Westchester, PA) namely D10-acenaphthalene, D12-chrysene, D10-phenanthrene and D12-perylene was used as an internal standard. The chemical structures of these internal standards are shown in Figure 2.

Soxhlet extraction

10 g of fresh fish fillet was homogenized in a mortar with about 10 g of Na_2SO_4 (Wang et al., 1999) 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 (US EPA, 1994) using 150 ml dichloromethane for 16 h. 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 (Mottier et al., 2000). This procedure was able to remove the very polar lipids off the extract as can be observed from Figures 3 and 4. The lipids, being very polar, were adsorbed at the

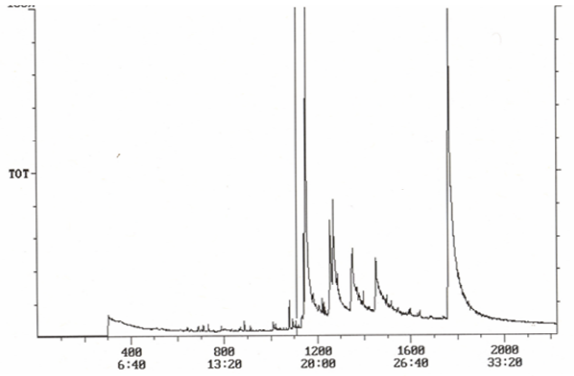

Figure 3. Total ion chromatogram of a fish extract without clean up.

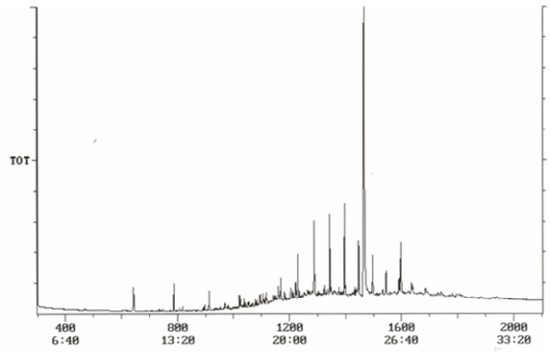

Figure 4. Total ion chromatogram of a fish extract after clean up.

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 and 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°C and 250°C, respectively. All injection volumes were 1 µl in the splitless mode. The column temperature was initially held at 70°C for 4 min, ramped to 300°C at a rate of 10°C/min, and then held at 300°C for 10 min (US EPA, 1999). The mass spectrometer was used in electron ionization mode and all spectra were acquired using a mass range of 50 – 400 m/z and automatic gain control (AGC).

RESULTS AND DISCUSSION

Chromatographic study

GC conditions were set using conditions similar to those in related studies (US EPA, 1999). The column temperature program gave baseline separation of the target compounds in a reasonable amount of time as shown in Figure 5, which depicts a chromatogram of a standard mixture of PAH containing 16 target compounds.

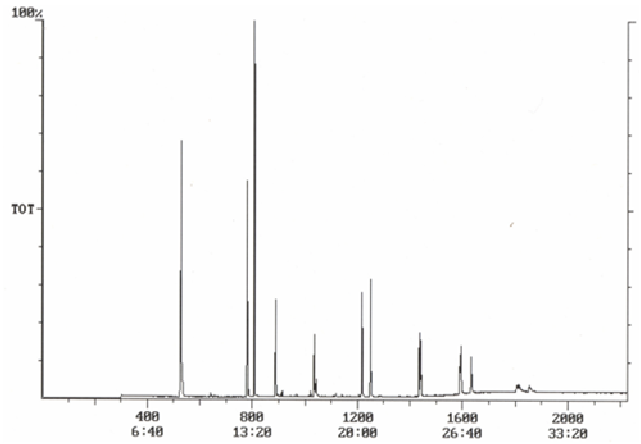

Figure 5. The chromatogram of 16 PAH standards.

Recovery studies of PAH from the sample

To evaluate the extraction efficiency for the target compounds, recovery studies were carried out using 4 isotopically labeled PAHs, namely D10-acenaphthene, D10-phenanthrene, D12-chrysene and D12-perylene, to represent 2- and 3-, 4-, 5- and 6-ring PAHs, respectively. The percent recoveries ranged from 90.58% to 118%, and demonstrate no significant losses of analytes during the sample workup process.

Analytical characteristics of the method

Calibration curves were obtained using a series of varying concentrations of a multi-component standard containing each of the 16 PAHs. All 16 calibration curves were linear, with correlation coefficients from the linear regression ranging from 0.994 to 1.000. Relative standard deviations from replicate analyses of the same standards (n = 3) were mostly below 10% as shown in Table 1. Limits of detection and quantitation (LODs and LOQs) are provided in Table 2. The lowest LOD was 0.02 µg/ml for lower molecular weight compounds while indeno(1,2,3-cd)pyrene has the highest at 1.7 µg/ml. Table 3 and 4 show the retention time and major ions for 16 PAHs used in the quantification and the internal standards respectively.

Identification and quantification

The PAHs in the sample were identified by a combination

Table 1. Calibration parameters of the PAH compounds.

Compound	Linear range (µg/ml)	Slope	Intercept	Regression coefficient	RSD %
Naphthalene	0.503 - 5.033	3.28	1.143	0.994	4.76
Acenaphthylene	0.387 - 3.888	6.93	0.074	0.997	1.7
Acenaphthene	0.519 - 5.193	4.653	0.342	0.996	0.89
Flourene	0.119 - 1.188	4.024	0.084	0.997	6.2
Phenanthrene	0.086 - 0.855	5.063	0.105	0.997	7.17
Anthracene	0.020 - 0.198	5.272	0.01	0.997	4.92
Flouranthene	0.191 - 1.910	6.108	0.243	0.998	2.48
Pyrene	0.212 - 2.118	6.269	0.664	0.998	4.4
Benz[a]anthracene	0.102 - 1.023	5.354	-0.134	0.999	5.36
Chrysene	0.092 - 0.918	5.388	0.089	0.996	4.26
Benzo[b]fluoranthene	0.104 - 1.043	10.249	-0.159	0.997	1.69
Benzo[k]fluoranthene	0.118 - 1.180	13.24	-0.417	0.999	2.71
Benzo[a]pyrene	0.123 - 1.228	6.884	-0.411	0.995	2.11
Benzo[ghi]perylene	0.354 - 0.885	1.377	-0.167	0.995	10.16
Dibenz[a,h]anthracene	0.368 - 0.920	0.922	-0.108	0.997	15.79
Indeno[1,2,3-cd]pyrene	0.428 - 1.070	1.307	-0.122	1	4.77

Table 2. List of LODs and LOQs for the 16 PAHs.

Compound	Mol. mass	No. of rings	Ret. time (min)	LOD (µg/ml)	LOQ (µg/ml)
Naphthalene	128	2	8.46	0.06	0.2
Acenaphthylene	152	3	13	0.02	0.06
Acenaphthene	154	3	13.26	0.02	0.06
Flourene	166	3	14.49	0.02	0.06
Phenanthrene	178	3	17.14	0.03	0.09
Anthracene	178	3	17.22	0.02	0.06
Flouranthene	202	4	20.16	0.04	0.12
Pyrene	202	4	20.49	0.04	0.12
Benz[a]anthracene	228	4	23.55	0.06	0.2
Chrysene	228	4	24	0.06	0.2
Benzo[b]fluoranthene	252	5	26.3	0.1	0.3
Benzo[k]fluoranthene	252	5	26.35	0.15	0.5
Benzo[a]pyrene	252	5	27.18	0.15	0.5
Benzo[ghi]perylene	276	6	30.06	0.75	2.5
Dibenz[a,h]anthracene	278	6	30.17	0.9	2.7
Indeno[1,2,3-cd]pyrene	276	6	30.55	1.7	5

of a retention time match and mass spectral match against the calibration standards. Quantitation was performed by the method of internal standardization as shown in Figures 6 and 7. D10-acenaphthalene was used as the internal standard for naphthalene, acenaphthylene, acenaphthene and fluorine. D10-phenanthrene was used as the internal standard for phenanthrene, anthracene, fluoranthene and pyrene. D-chrysene was used for benz[a]-anthracene and chrysene. D12-perylene was used for the rest of the PAHs.

Determination of PAH in the samples

Figures 8 to 11 show the distribution of PAHs in different fish species. Figure 12 compares the four species for PAH distribution. Table 5 provides a summary of their concentrations in the samples. All 16 PAHs were found to be present in significant amount, indicating a high level of contamination. The PAH concentration did not show any particular trend, since both fresh water and brackish water species have concentration close to one another. It

Table 3. List of major ions for the 16 PAHs.

Compound	m/z
Naphthalene	128, 115, 102, 87, 75, 63, 51
Acenaphthylene	152, 126, 98, 87, 76, 63, 50
Acenaphthene	154, 126, 102, 87, 77, 63, 50
Flourene	166, 139, 115, 83, 63, 50
Phenanthrene	178, 152, 126, 111, 99, 89, 76, 63, 50
Anthracene	178, 152, 126, 89, 76, 63,
Flouranthene	202, 174, 150, 122, 101, 87, 74, 50
Pyrene	202, 174, 150, 101, 88, 74, 50
Benz[a]anthracene	228, 200, 150, 113, 88, 63, 50
Chrysene	228, 202, 176, 150, 113, 101, 63
Benzo[b]fluoranthene	252, 224, 174, 150, 126, 113, 86
Benzo[k]fluoranthene	252, 224, 198, 150, 126, 74
Benzo[a]pyrene	252, 225, 161, 126, 74
Benzo[ghi]perylene	276, 248, 225, 207, 191, 138, 125, 97, 73
Dibenz[a,h]anthracene	278, 248, 225, 207, 191, 138, 125, 83, 73, 57
Indeno[1,2,3-cd]pyrene	276, 248, 225, 207, 191, 138, 111, 97, 73, 57

Table 4. List of major ions for the four internal standards.

Compound	m/z
D-acenaphthene	164, 132, 108, 84, 66, 51
D-phenanthrene	188, 160, 132, 94, 80, 66, 51
D-chrysene	240, 208, 156, 120
D-perylene	264, 236, 207, 180, 132, 118, 86

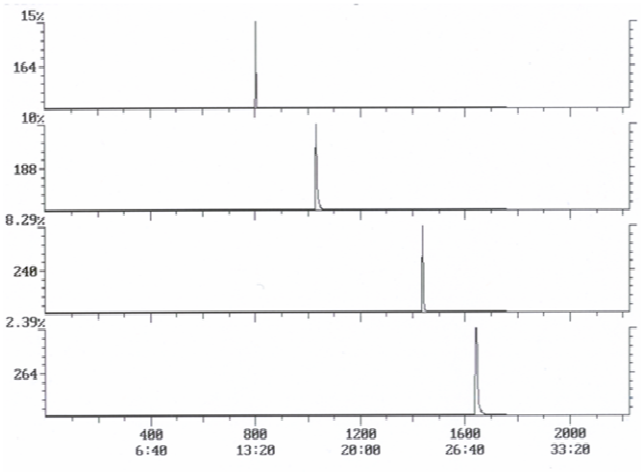

Figure 6. Selected ion chromatogram of the internal standard.

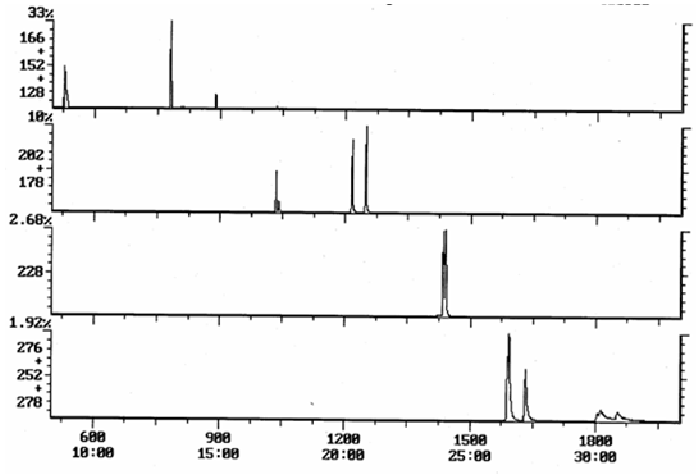

Figure 7. Selected ion chromatogram of the 16 EPA priority PAH.

was also easily seen that the 5- and 6-ring PAH are present in the highest concentrations. This could be explained by the fact that these PAHs are more resistant to degradation and removal in the fish and in the environment. Previous work shows that this same pattern is observed in the other environmental samples (water and sediment) in the region. Our result shows that the fish samples in the Niger Delta are highly polluted especially when compared with data from other parts of the world. The total PAHs in fish found in Puget Sound, Washington USA was below 200 ng/g (Landlot et al., 1987). In the Red Sea coast of Yemen, the concentration was 422.1 ng/g (DouAbul et al., 1997). In Phillip Bay, Victoria the total PAHs found in fish was just 55.7 ng/g (Nicholson et al., 1994) as against an average of 100 ug/kg obtained in this study.

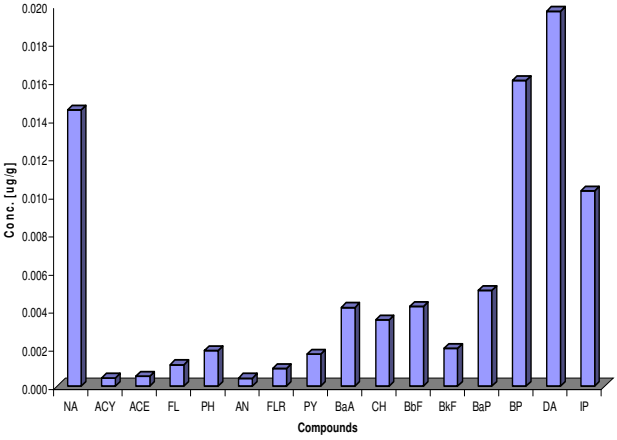

Figure 8. The PAH distribution in *P. obscura*.

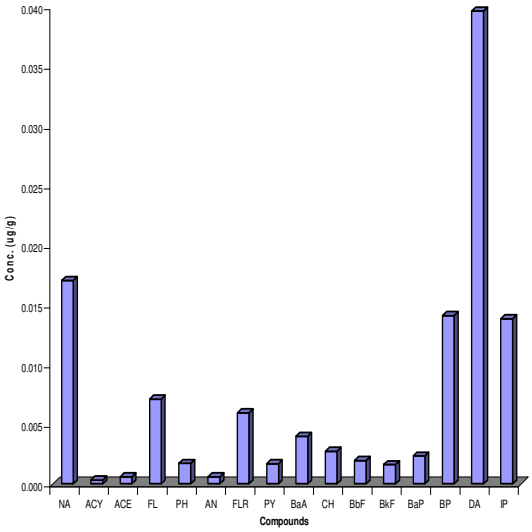

Figure 9. The PAH distribution in *C. gariepinnus*.

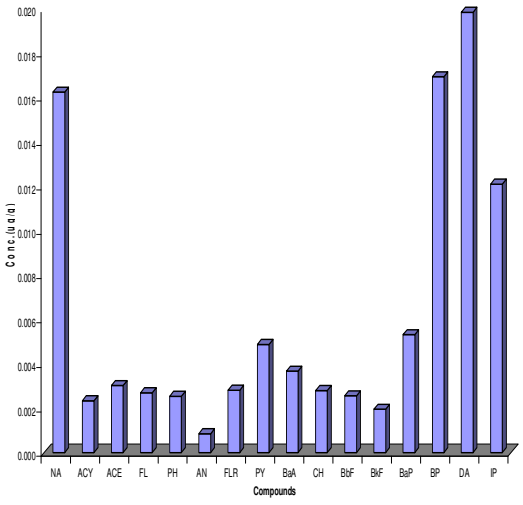

Figure 10. The PAH distribution in *L. dumerillii*.

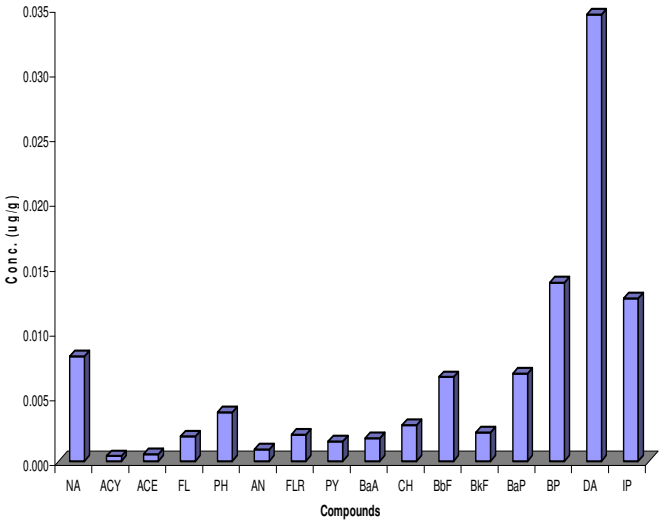

Figure 11. The PAH distribution in *P. elongates*

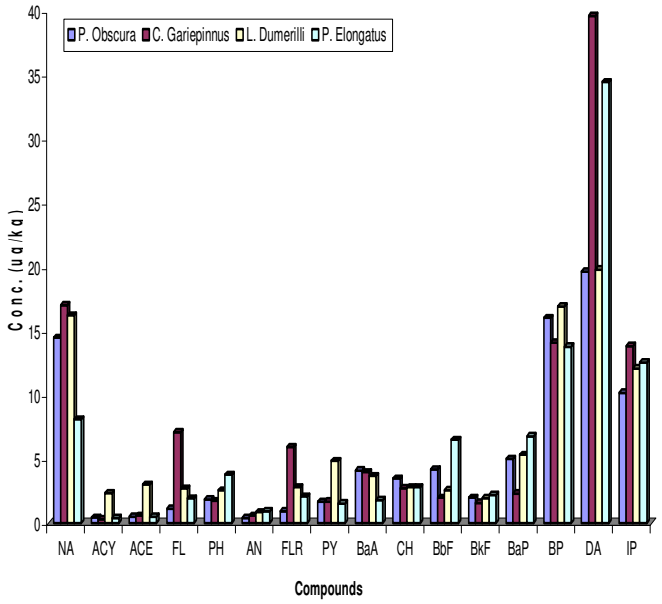

Figure 12. Comparison of the four fish species: Distribution of PAHs in different fish species

Conclusion

These results show a high level of PAHs in the fish samples that are consumed by the population. This suggests a significant risk when such contaminated fish represent a significant component of the diet. It was also discovered that samples absorbs PAH's in more or less the same pattern but interspecies differences can only lead to just slightly different rates of absorption and ability to accumulate these samples in fish tissues.

Table 5. The PAH concentration of different species of fish studied in μg/kg.

Compound	*P. obscura*	*C. gariepinnus*	*L. dumerillii*	*P. elongatus*
Naphthalene	14.50	17.03	16.24	8.10
Acenaphthylene	0.42	0.31	2.34	0.40
Acenaphthene	0.50	0.59	3.02	0.50
Flourene	1.12	7.10	2.69	1.92
Phenanthrene	1.86	1.72	2.54	3.77
Anthracene	0.41	0.59	0.84	0.93
Flouranthene	0.92	5.95	2.81	2.08
Pyrene	1.68	1.69	4.87	1.53
Benz[a]anthracene	4.12	3.98	3.67	1.79
Chrysene	3.48	2.72	2.80	2.79
Benzo[b]fluoranthene	4.19	1.97	2.56	6.50
Benzo[k]fluoranthene	1.98	1.60	1.96	2.20
Benzo[a]pyrene	5.01	2.32	5.32	6.78
Benzo[ghi]perylene	16.04	14.11	16.93	13.80
Dibenz[a,h]anthracene	19.67	39.64	19.83	34.48
Indeno[1,2,3-cd]pyrene	10.20	13.84	12.09	12.57

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