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Full Length Research Paper

Determination of polynuclear aromatic hydrocarbons (PAHs) in selected water bodies in the Niger Delta

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Several water bodies in the Niger Delta region of Nigeria where extensive crude oil production activities take place were analyzed for the presence of 16 US EPA priority polynuclear aromatic hydrocarbons (PAHs) namely: naphthalene, acenaphthylene, acenaphthene, fluorine, 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. The concentrations ranged from as low as 1.95 ug/L for relatively clean stream with practically no crude oil activity to 10.9 ug/L for the most polluted. The analysis was carried out using GC/MS. The quantitation was done by means of internal standardization using four isotopically labeled internal standards namely acenaphthene-$_{d10}$, chrysene-d_{12}, phenanthrene-$_{d10}$ and perylene-d_{12}. High molecular mass PAHs such as benzo(ghi)perylene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene were mostly absent confirming low water solubility of these compounds and carcinogenic PAHs were general lower in concentration than the non carcinogenic ones.

Key words: Polynuclear Aromatic Hydrocarbons, Crude Oil, GC/MS, Niger Delta

INTRODUCTION

The contamination of the environment by polynuclear aromatic hydrocarbons (PAHs) is becoming a rising environmental concern. They have a widespread distribution in the environment and the carcinogenicity and mutagenicity of several of these compounds have been proven (Simko, 2002; Koyano et al., 2001; Liu and Korenga, 2001; Alonge, 1988). In 2001 PAHs were ranked the ninth most threatening compounds to human health (King et al., 2002). Several epidemiological studies on PAHs especially among workers exposed to these compounds in a number of countries have been carried out (Grimmer et al., 1988). PAHs comprise the largest class of chemical compound known to be cancer-causing agents and are included in the European Union and United States Environmental Protection Agency (EPA) priority pollutant list due to their mutagenic and carcinogenic properties.

PAHs consist of several hundred compounds containning two or more condensed rings. Among the several hundred different PAHs already identified, sixteen are considered as priority because they are supposed to be more harmful than the others; there is more information available on them and there is a greater possibility of people being exposed to them. Both natural and anthropogenic sources contribute PAHs to the environment. But crude oil and other petroleum based products have been found to contribute significant amount of PAHs to the environment. Other sources of PAHs in the environment include natural fires, volcanic eruptions, thermal geological reactions, industrial processes (aluminium production, iron and steel production, foundries), transportation, burning (e.g. forest, straw, agriculture, cooking), waste incineration, combustion of fossil fuel, exhausts from vehicles, tobacco smoke, domestic heating using wood, coal, and mineral oil etc (Nieva-Cano et al., 2001; Grova et al., 2002; Guillen et al., 2000; Anyakora et al., 2005).

Since the discovery of crude oil and subsequent exploration and exploitation of crude oil in the Niger Delta region of Nigeria several decades ago, there have been incessant spillages with causes ranging from accident to sabotage. All these contribute to the amount of PAHs in the water bodies of this environment. This study tries to

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Table 1. A list of the surrogate standards and the corresponding PAHs they represent.

Acenaphthene-$_{d10}$	Phenanthrene-$_{d10}$	Chrysene-$_{d12}$	Perylene-$_{d12}$
Naphthalene	Phenanthrene	Benz(a)anthracene	Benzo(b)fluoranthene
Acenaphthene	Anthracene	Chrysene	Benzo(k)fluoranthene
Acenaphthylene	Fluoranthene		Benzo(a)pyrene
Fluorene	Pyrene		Benzo(ghi)perylene
			Dibenz(a,h)anthracene
			Indeno(1,2,3-cd)pyrene

evaluate the extent of PAH contamination in some selected water bodies in this region.

MATERIALS AND METHOD

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. A PAH standard mixture (NIST, Baltimore, MD) containing naphthalene, acenaphthylene, acenaphthene, fluorine, 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. 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.

Collection of samples

Water samples were collected from nine different water bodies in the Niger Delta (hereafter refer to as samples 1 to 9). The samples were collected from locations that have some history of oil pollution or are close to some oil production facilities. The samples were collected with sterilized 500 ml sample bottles at a depth between one and two meters. These samples were acidified at point of collection with concentrated hydrochloric acid to render inactive any microorganism that may cause biodegradation of the samples. The sample bottles were ember coloured to prevent UV light from effecting degradation of the analytes. The samples were transported to the labouratory at a temperature below 10^{o}C and stored at that temperature until ready for use.

Preparation of standard solution

Five standard solutions each containing 16 target compounds were prepared by diluting the standard mix (1647 mix from NIST) to desired concentrations with HPLC grade dichloromethane. To all these solution were added 0.5 µg each of the four internal standards namely acenaphthene-$_{d10}$, chrysene-d_{12}, phenanthrene-$_{d10}$ and perylene-d-$_{12}$. These were transferred to a capped and sealed vial until ready for analysis.

Extraction

The extraction of the samples was carried out by liquid-liquid extraction method (US EPA, 1994). The apparatus for this consisted of a 100 ml volume separating funnel mounted on a retort stand. The separating funnel was thoroughly washed and dried over night in a muffle furnace at an elevated temperature. Prior to use the funnel was rinsed vigorously with dichloromethane for several minutes. This was removed and allowed to drain and dry completely in fume cupboard. 20 ml of water sample to be extracted was transferred to the separating funnel and to this was added 20 ml of dichloromethane. This was shaken vigorously for 2 min and allowed to separate and settle. After 10 min the organic layer was removed and the process repeated with the aqueous layer twice.
The three portions of the organic phase were combined and evaporated to 1ml volume using a rotary evaporator.

Recovery studies

Prior to extraction, four surrogate standards were added to the sample. A surrogate is a chemical compound not expected to occur in the sample under study. This is used to monitor for unusual matrix effect, gross sample processing error etc. Four surrogate standards were used to monitor the recovery of different target compounds. The surrogate standards used include acenaphthene-$_{d10}$, chrysene-d_{12}, phenanthrene-$_{d10}$ and perylene-d_{12}. The compounds they represented are as shown in Table 1.

The samples were subjected to the same extraction procedure as described above. 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% (US EPA, 1999).

Calibration

Several dilutions of the standard PAH mixture made 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 for the sixteen compounds were determined by triplicate of each analysis. The r^2 was determined for each compound was using excel formula software.

Analysis by GC/MS

GC/MS analysis was carried out on a Finnigan Magnum instrument equipped with a CTC A200S autosampler and a 30 µm, 0.25 ID DB-5 MS fused silica capillary column (J & W Scientific, Folson CA).

Table 2. Chromatographic characteristic of the target compounds.

Compound	Ret. Time (mins)	Working range	Major Peak ion	Internal standard	regression coefficient	RSD %	LOD (ug/ml)	LOQ (ug/ml)
Naphthalene	8.46	0.503 - 5.033	128	Acenaphthene-$_{d10}$	0.994	4.76	0.06	0.20
Acenaphthylene	13.00	0.387 - 3.888	152	Acenaphthene-$_{d10}$	0.997	1.70	0.02	0.06
Acenaphthene	13.26	0.519 - 5.193	154	Acenaphthene-$_{d10}$	0.996	0.89	0.02	0.06
Fluorene	14.49	0.119 - 1.188	166	Acenaphthene-$_{d10}$	0.997	6.20	0.02	0.06
Phenanthrene	17.14	0.086 - 0.855	178	Phenanthrene-$_{d10}$	0.997	7.17	0.03	0.09
Anthracene	17.22	0.020 - 0.198	178	Phenanthrene-$_{d10}$	0.997	4.92	0.02	0.06
Fluoranthene	20.16	0.191 - 1.910	202	Phenanthrene-$_{d10}$	0.998	2.48	0.04	0.12
Pyrene	20.49	0.212 - 2.118	202	Phenanthrene-$_{d10}$	0.998	4.40	0.04	0.12
Benz(a)anthracene	23.55	0.102 - 1.023	228	Chrysene-$_{d12}$	0.999	5.36	0.06	0.20
Chrysene	24.00	0.092 - 0.918	228	Chrysene-$_{d12}$	0.996	4.26	0.06	0.20
Benzo(b)fluoranthene	26.30	0.104 - 1.043	252	Perylene-$_{d12}$	0.997	1.69	0.10	0.30
Benzo(k)fluoranthene	26.35	0.118 - 1.180	252	Perylene-$_{d12}$	0.999	2.71	0.15	0.50
Benzo(a)pyrene	27.18	0.123 - 1.228	252	Perylene-$_{d12}$	0.995	2.11	0.15	0.50
Benzo(ghi)perylene	30.06	0.354 - 0.885	276	Perylene-$_{d12}$	0.995	10.16	0.75	2.50
Dibenz(a,h)anthracene	30.17	0.368 - 0.920	278	Perylene-$_{d12}$	0.997	15.79	0.90	2.70
Indeno(1,2,3cd)pyrene	30.55	0.428 - 1.070	276	Perylene-$_{d12}$	1.000	4.77	1.70	5.00

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 and 250 ^{o}C, respectively. All injection volumes were 1 µl in the splitless mode. The column temperature was initially held at 70^{o}C for 4 min, ramped to 300^{o}C at a rate of 10^{o}C/min, and then temperature was held at 300^{o}C for 10 min. 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).

Identification and quantitation

Identification of the compounds was based on the retention time match and mass spectra match against the calibration standards. Quantitation was performed by the method of internal standardization using acenaphthene-$_{d10}$, chrysene-d_{12}, phenanthrene-$_{d10}$ and perylene-d_{12}. Acenaphthene-$_{d10}$ was used as the internal standard for naphthalene, acenaphthylene, acenaphthene and fluorene. Phenanthrene-$_{d10}$ was used as the internal standard for phenanthrene, anthracene, fluoranthene and pyrene. Chrysene-d_{12} was used for benz(a)anthracene and chrysene. Perylene-d_{12} was used for benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, dibenz(a,h)anthracene and indeno(1,2,3-cd) pyrene. The quantitation was based on the ratio of the peak height of the quan ion to that of the corresponding internal standard. The possibility of selected ion chromatogram enabled us detect the target ion without ambiguity despite the complexity of the samples.

RESULTS AND DISCUSSION

Determination of the analytical characteristics

GC conditions were set to give a baseline separation of the target compounds in a reasonable time of less than 35 min. This was achieved by setting the chromatographic conditions as those described above. Calibration curves were obtained using a series of varying concentrations of a multi-component standard containing each of the 16 PAHs. The curves were obtained by plotting target analyte/internal standard peak height ratio against concentration. A linear relationship was obtained with correlation coefficients from the linear regression of 0.994 and above. Other analytical parameters for the chromatographic method such as relative standard deviations (RSD), limits of detection (LOD), limits of quantitation (LOQ) etc are provided in Table 2.

Evaluation of the extraction efficiency

To evaluate the extraction efficiency for the target compounds, recovery studies were carried out using 4 isotopic PAHs (acenaphthylene-d_{10}, phenanthrene-$_{d10}$, chrysene-$_{d12}$ and perylene-$_{d12}$). Acenaphthylene-d_{10} served as a surrogate for four compounds namely, naphthylene, acenaphthylene, acenaphthene and fluorene. These four compounds have molecular masses close to that of the surrogate (164) and have chemical characteristics similar to that of acenaphthene-$_{d10.}$ Phenanthrene-$_{d10}$ was used as a surrogate for phenanthrene, anthracene, fluoranthene and pyrene. Both the molecular masses and structures of these compounds are significantly similar to that of phenanthrene-$_{d10}$.

Chrysene-$_{d12}$ was used as a surrogate for both chrysene and benz(a)anthracene. As in the cases above chrysene-$_{d12}$ is very suitable because of significant similarities in their properties. Perylene-$_{d12}$ was used as a surrogate for the six remaining compounds namely, benzo-

Table 3. The list of ascribed percentage recove-ries in the sample.

Compound	Recovery
Naphathalene	71.12%
Acenaphthylene	71.12%
Acenaphthene	71.12%
Flourene	71.12%
Phenanthrene	91.94%
Anthracene	91.94%
Flouranthene	91.94%
Pyrene	91.94%
Benz(a)anthracene	61.62%
Chrysene	61.62%
Benzo(b)flouranthene	64.78%
Benzo(k)flouranthene	64.78%
Benzo(a)pyrene	64.78%
Benzo(ghi)perylene	64.78%
Dibenz(a,h)anthracene	64.78%
Indeno(1,2,3-cd)pyrene	64.78%

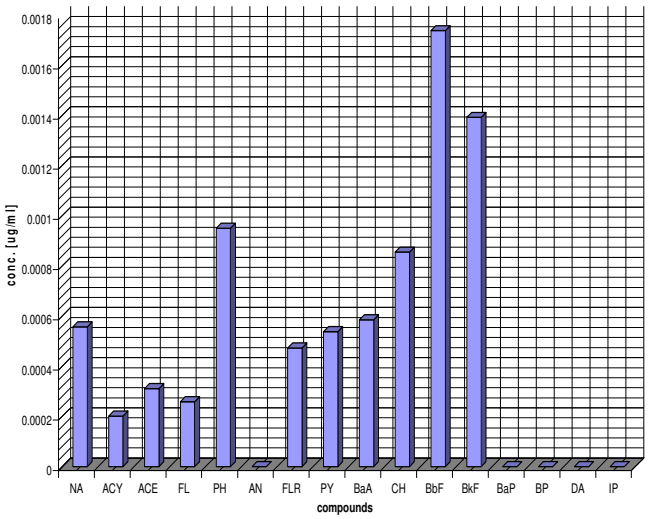

Figure 1. PAH distribution in sample 1.

(b)fluoranthene, benzo(k)fluoranthene, benzo(a) pyrene, benzo(ghi)perylene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene. One similarity that exists among these compounds is the possession of 5 or 6 aromatic rings. With this we can propose an approximate recovery for the studied samples as shown in Table 3.

Determination of PAH in contaminated sample

PAHs were determined in the samples using the esta-blished method and procedures as described above. Most of the studied water samples did not contain high molecular weight PAHs such as benzo(ghi)perylene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene. This is due to the low water solubility of these compounds (Anyakora et al., 2004). These compounds are rarely found in water samples unless there is a presence of dissolved solids that have these compounds attached to them. Figures 1 to 9 show the PAH distribution in these samples.

Implication of results

PAHs have received a considerable attention in recent years because of their carcinogenic properties. According to the World Health Organization study in 1997, the concentration of individual PAHs in surface and coastal waters are generally in the neighborhood of 0.05 µg/L (WHO, 1998) and concentration above this point indica-tes some contamination, also a study carried out by the World Health Organization in 1993 revealed that Benzo (a) pyrene concentration of 0.7 µg/L corresponds to an excess lifetime cancer risk of 10^{-5} (WHO, 1998). Benzo (a) pyrene is the most studied PAH because it is the most dangerous. It is used as an index for the level of PAH contamination because of this reason. Figure 10 shows

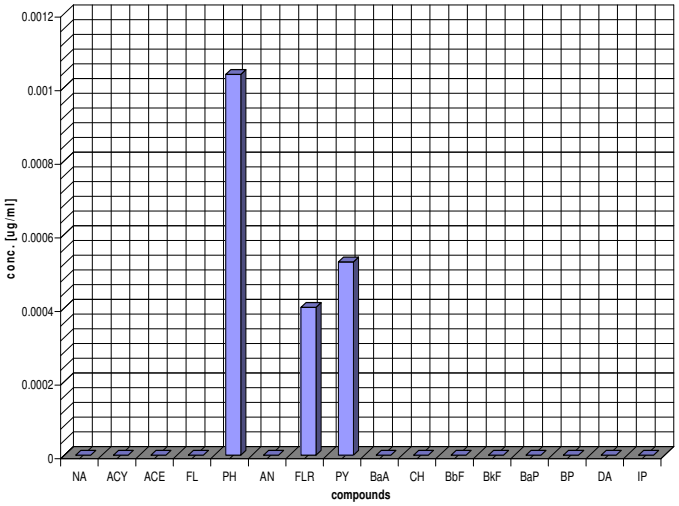

Figure 2. PAH distribution in sample 2.

the profile of benzo (a) pyrene in the studied samples in comparison with the average concentration of those from the Red Sea Coast of Yemen (DouAbul et al., 1997), a comparable environment with crude oil exploration. 60% of the samples were above WHO limits but fewer number of samples exceeded what was obtained in a similar environment as can bee seen from Figure 10.

Carcinogenic and non carcinogenic PAHs

Based on qualitative classification of PAH carcinogenicity (WHO, 1998), we can divide the sixteen priority PAHs (our target compounds) into two categories. The first category contains PAHs having sufficient or limited evid-ence for carcinogenicity namely benzo(a)pyrene, benz(a)

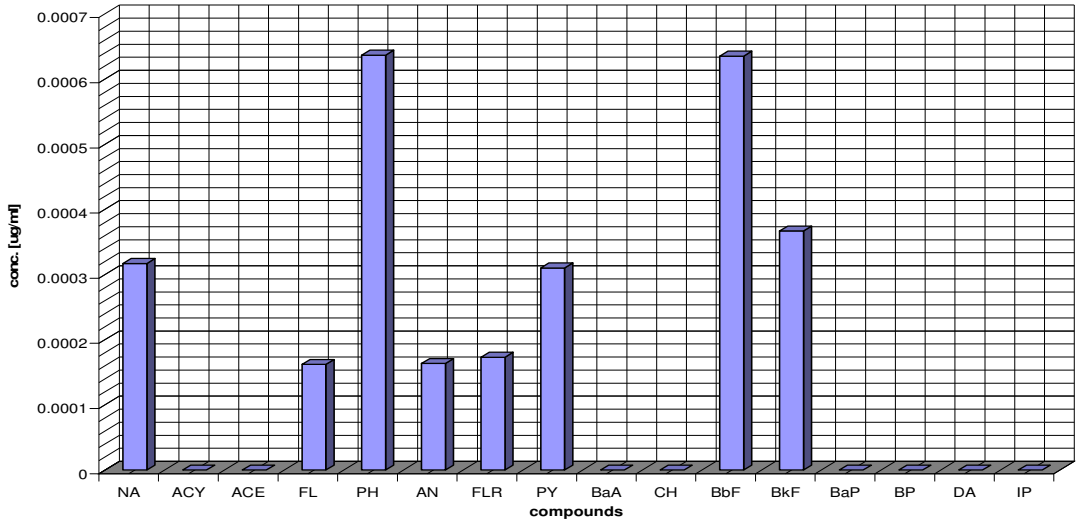

Figure 3. PAH distribution in sample 3.

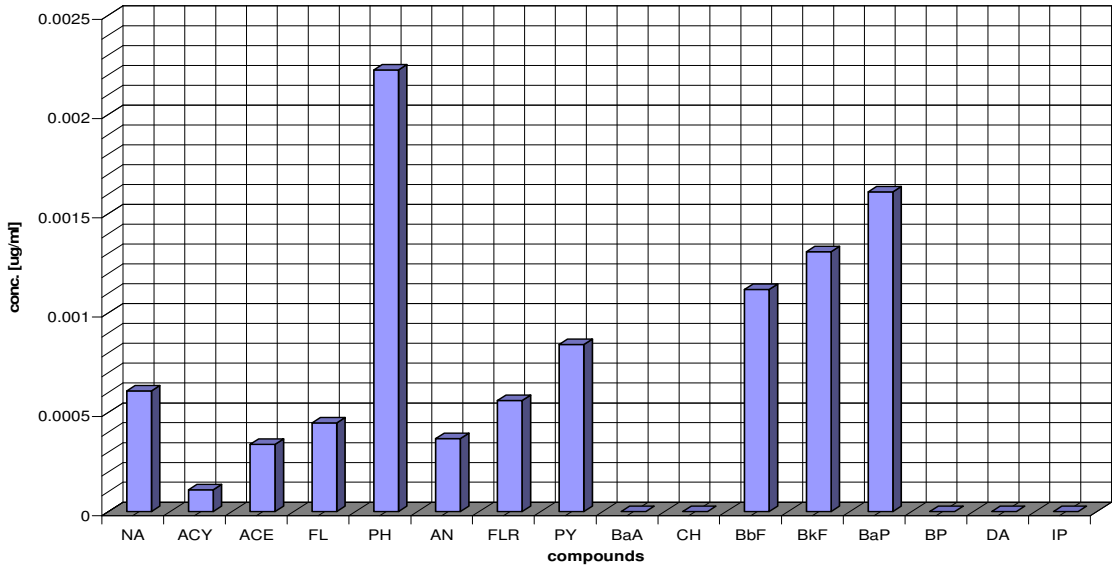

Figure 4. PAH distribution in sample 4.

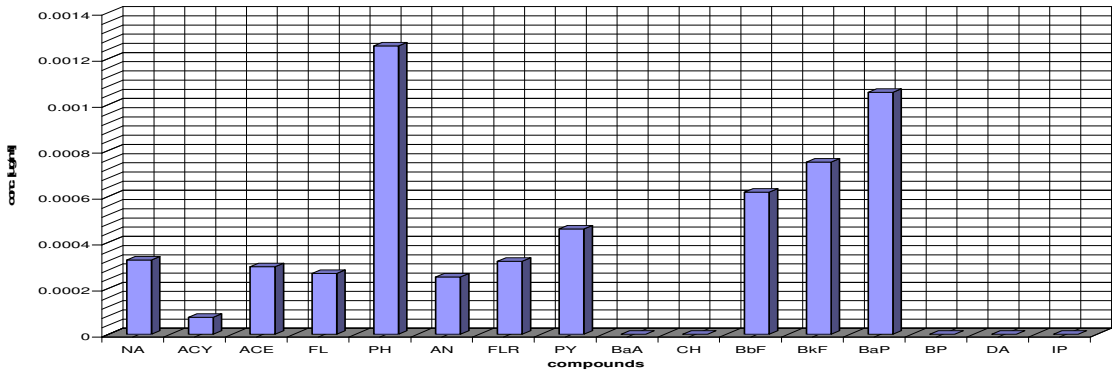

Figure 5. PAH distribution in sample 5.

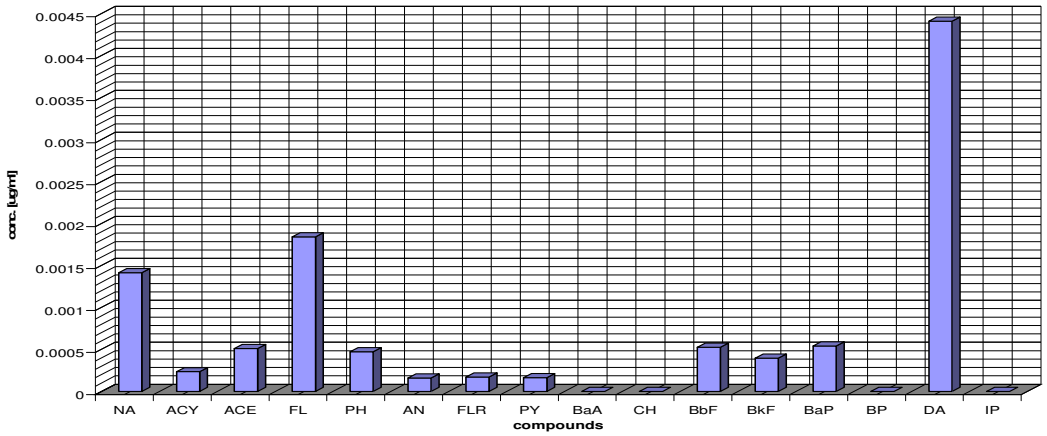

Figure 6. PAH distribution in sample 6.

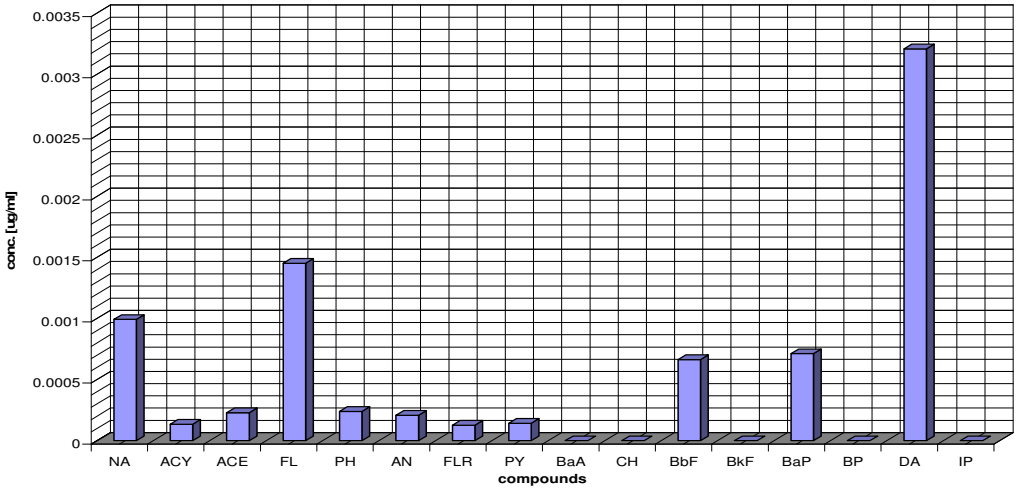

Figure 7. PAH distribution in sample 7.

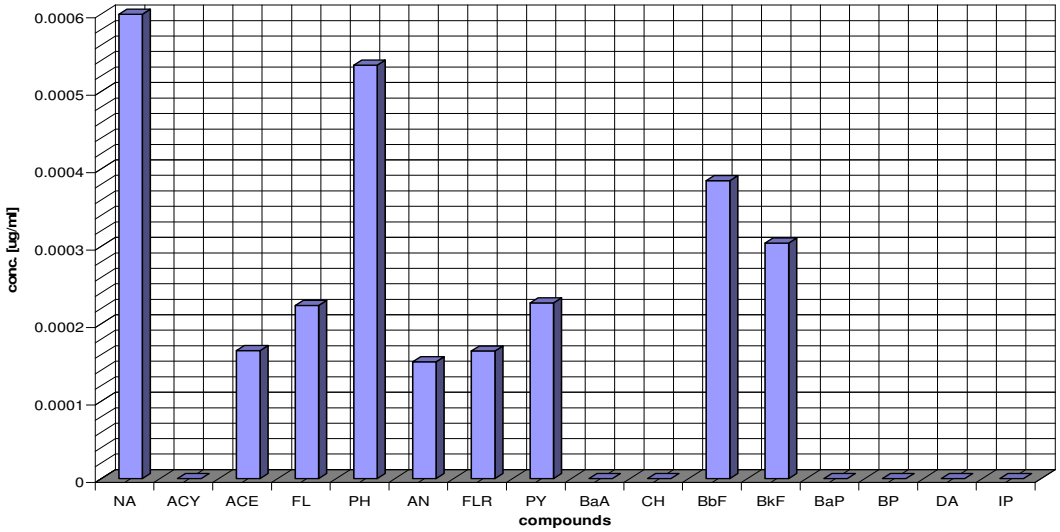

Figure 8. PAH distribution in sample 8.

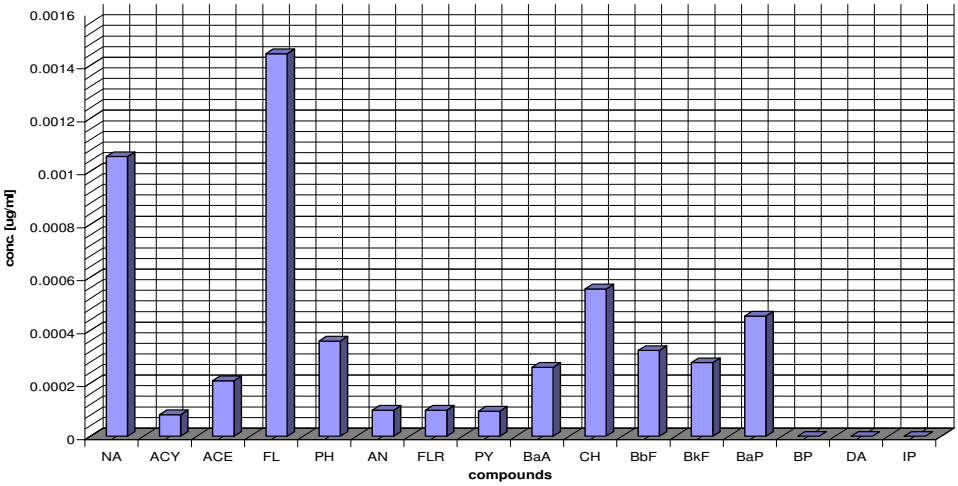

Figure 9. PAH distribution in sample 9.

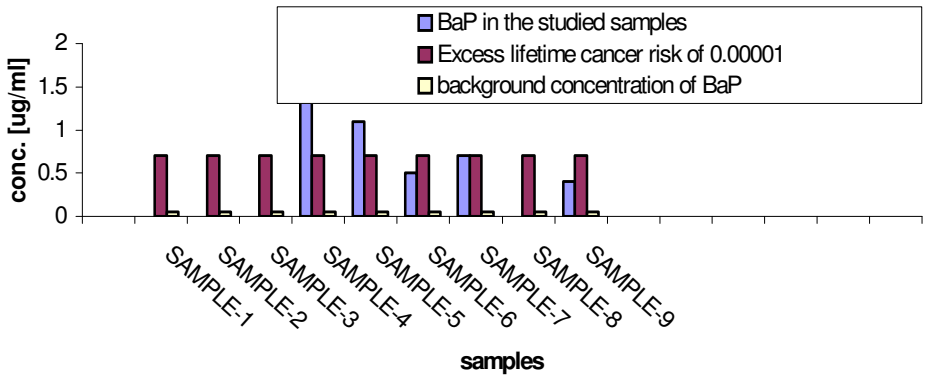

Figure 10. Benzo(a)pyrene profile in the studied water samples.

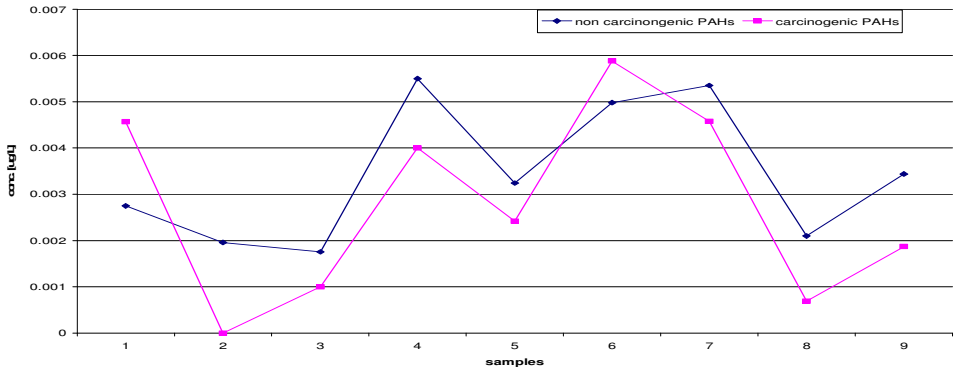

Figure 11. The profile of carcinogenic and non-carcinogenic PAHs in the studied samples.

anthracene, chrysene, dibenz(a,h)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, indeno (1,2,3-cd)pyrene and benzo(ghi)perylene. The second categories are the PAHs that have insufficient or no evidence for carcinogenicity namely naphthalene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, acenaphthylene and acenaphthene. Figure 11 shows the profile of total carcinogenic PAHs and that of non carcinogenic

PAHs. The total carcinogenic PAHs were generally lower in the studied samples with very few exceptions as can be seen from figure 11. Even though most of the carcinogenic PAHs have greater resistance to microbial degradation (Yun et al., 2003), their limited water solubility is a major reason for their reduced concentration in the studied samples.

Conclusion

The data available from our studies show that there is relatively high level of PAHs in this environment exceeding the WHO recommended maximum value for safety. This suggests significant risk of cancer to the people of this environment. Considering limited water solubility of PAHs, it is expected that significantly higher concentrations will be detected in other lipid rich samples of this environment.

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