Polycyclic aromatic hydrocarbons in water samples from a Nigerian bitumen seepage: Gas chromatography- mass spectrometry quantification

A. U. Itodo*, R. Sha’Ato and M. I. Arowojolu

Department of Chemistry, Federal University of Agriculture, Makurdi, Nigeria

Abstract

Bitumen exploration was flagged off at Irele, a Nigerian field in 2003. The relationship between the seepage pollutants and receiving water bodies is the focus of this study. We hereby present the polycyclic aromatic hydrocarbons (PAHs) level in two categories of bitumen contaminated waters; source (RS) and delivery (RD) points. Results were compared with uncontaminated water (RC) as control experiment. The PAHs were extracted by Liquid-Liquid extraction using dichloromethane and analyzed by Gas chromatography with mass spectrometer detector (GC-MS). The mean concentration of PAHs was 8.39, 3.93 and 0.57 ppb for source, delivery point and control respectively. The Benzo[ghi]pyrene concentrations obtained were higher than 1-10 ppb in most samples, indicating anthropogenic and petrogenic sources of the pollutants. Most of the PAH concentrations obtained were within natural concentrations. However, Benzo (e) pyrene and Indenol (1, 2, 3 – cd) pyrene exceeded the expected limit. The predominance of 4-6 member ring showed that the PAHs were more of pyrolytic than mixed sources. Method validation gave the Limit of Detection (LOD) and Limit of Quantification values in the range of 0.03-0.12 and 0.10-0.61 respectively. The Pearson product moment correlation (PPMC) coefficient ($r$) was used to determine the interactions of the PAHs detected with parametric factors. All the physicochemical parameters studied except pH shows positive correlation. Statistical test at P <0.05 indicated that the difference in PAHs concentrations is not significant when compared with maximum acceptable PAHs concentration (MAC) for water, which is an indication that the water is safe.

Keywords: Polynuclear; Bituminous; Seepage; Environment; Pollution and anthropogenic petrogenic

Introduction

Water pollution occurs when a body of water receives excessive amounts of materials called contaminants, such that the quality of the water becomes adversely affected (Olabemiwo et al., 2011). Organic polluted water causes aquatic-toxicological effects which are deleterious to aquatic life (Carls et al., 1999).

Bitumen can be obtained from various sources. It occurs naturally, but for most purposes it is petroleum on which the world relies for the supplies of bitumen. Its composition is very complex, containing a mixture of high boiling point range of compounds and molecules with a relatively low hydrogen-to-carbon ratio (Yoon et al., 2009). Bitumen contains very small quantities of PAHs having 4–6 condensed rings. When bitumen is in its natural state, the PAHs are not bio-available, but when it is used in a mixture with other solvents that can dissolve it, the PAHs can be released into the environment (Fouial–Djebbar et al., 2010).

Polycyclic Aromatic Hydrocarbons (PAHs) form a major component of organic contaminants in the environment. They are available is soil and water, upon which humans and animals depend for their survival. They have a unique characteristic of being carcinogenic, mutagenic and teratogenic that threatens human health (Fagbote and Olanipekun, 2013). The simplest PAHs, as defined by the International Union of Pure and Applied Chemistry (IUPAC), are phenanthrene and anthracene, which both contain three fused aromatic rings.
Smaller molecules, such as benzene, are not PAHs. Naphthalene, which consists of two coplanar six-membered rings sharing an edge, is another aromatic hydrocarbon which by formal convention, is not a true PAH, though is referred to as a bicyclic aromatic hydrocarbon (Rengarajan, et al., 2015).

Table I. presents 8 studied PAHs out of the 16 EPA priority PAHs and their molecular structures

| PAH                    | Abbreviation | Molecular Structure |
|------------------------|--------------|--------------------|
| Benz(a)anthracene      | BaA          | ![BaA](image)      |
| Benzo(e)pyrene         | BeP          | ![BeP](image)      |
| Benzo(b)fluoranthene   | BbFA         | ![BbFA](image)     |
| Benzo(ghi)perylenes    | BghiP        | ![BghiP](image)    |
| Fluorene               | FLR          | ![FLR](image)      |
| Indeno(1,2,3-c,d)pyrene| IP           | ![IP](image)       |
| Phenanthrene           | PHE          | ![PHE](image)      |
| Pyrene                 | PYR          | ![PYR](image)      |

Source: [7]

Other sources of PAHs are Surface runoff from roads and fields (Oyo-Ita, et al., 2011), Pollutants remaining in soils after flooding (Oleszczuk et al., 2003). Due to the adverse health properties of these compounds, maximum allowable concentrations (MACs) has been established by United States Agency for Toxic Substances and Disease Registry (US-ATSDR) for PAHs in soil and water, which calls for monitoring of these residues because of their ingestion potentials.

Kabzinski and Cyran (2002) reported total concentration of the 16 EPA Priority PAHs ranging from 64 – 8339 ng/L in the water samples collected from different locations in Lodz. Concentration of PAHs in lakes and rivers was reportedly linked to the high level of runoff and rain water at the beginning of the raining season. In a similar study, Fagbote and Olanipekun (2010) found the sum concentration of 16 EPA prioritized health threaten PAHs range from 0 - 0.12 ppm in the sediment of bitumen deposit of Agabiu, Nigeria using GC-MS. It was established that the high amount of PAHs in the rainy season may probably be due to rainfall and runoff. This research work therefore investigated the availability and amount of PAHs in water samples from Loda-Irele Bitumen seepage using GC-MS quantification. It is hoped that findings from this work will serve as reference background data on PAHs in water samples in Irele region as a pre-exploitation status record of the PAHs in the area and as part of data, essential in monitoring the extent of contamination by toxic substances.

**Sampling sites description**

Irele (6°29'0"N 4°52'0"E) Local Government Area in Ondo State, Nigeria (Figure 1) covers an area of 963 square...
kilometers, has a population of 145,166 with moderate temperature throughout the year, with a minimum around 24 °C and the maximum around 33 °C. Irele bitumen seepage (where bitumen exploration was flag off in the year 2003) spreads into lands used for agricultural purposes, reserve forest and rivers. Water samples from the source, delivery point and non bituminous (control) location were used in this study.

Materials and methods

Materials

Global positioning system (GPS), GC-MS and routine laboratory apparatus were employed in this research. All reagents used in the analysis were of analytical grade (AR). Two water samples; source (RS) and delivery (RD) points, at a distance of about 200 m apart were collected from river nearest to the bitumen seepage in the field using sterilized 2.5 L amber reagent bottles at a depth of 1-5 cm (Anyakora and Coker, 2006). Sample code, geographical coordinates and description are presented in Table II.

Table II. Sample codes, geographical coordinates and description

| S/N | Samples | Sample Codes | Geographical Coordinates | Description |
|-----|---------|--------------|---------------------------|-------------|
| 1   | Water   | RS           | 6.653225, 4.887798        | Water sample from the source of water around the bitumen seepage |
| 2   |         | RD           | 6.646865, 4.892376        | Water sample from the delivery point around the bitumen seepage |
| 3   |         | RC           | 6.370507, 4.939086        | Water sample from none bituminous region |

Sample treatment

Water samples were preserved by adding 2 mL concentrated hydrochloric acid at point of collection to render inactive any microorganism that may cause biodegradation of the samples (Anyakora and Coker, 2006), transported to the lab and stored below 4°C in a refrigerator prior to extraction.

Physicochemical analyses

Some physicochemical parameters were estimated according to procedures defined in the Standard Method for the analysis of water and wastewater. Those considered in this research are temperature, pH, electrical conductivity, Turbidity and total organic matter (TOM) of the water samples.

Temperature

The temperature was measured using mercury in glass thermometer immediately after sampling. Water sample (50 mL) was transferred into a beaker, the thermometer was inserted into the sample and reading on the thermometer was noted and recorded (Oyem et al., 2014).

Electrical conductivity

The conductivity of water samples was measured using DDS 307 conductivity meter at in the laboratory same day of sample collection. The meter was set to conductivity mode, standardized with distilled water in accordance with the instruction manual and readings were taken and recorded in µ S cm⁻¹ (Onojake, 2005).

Turbidity

The water samples’ turbidity was measured with Hach Laboratory Turbidimeter Model 2100 N. The sample cells were washed with deionized water and the sample (30 mL) was transferred into the cells. The sides of the cells were cleaned with a dry cloth. The power source of the turbidimeter was switch on and reading was taken in NTU (Odigure et al., 2005).

pH

A digital pH meter (PHS-3B uniscope pH meter) was used to measure pH of the water samples. Readings was recorded after the instrument gave stabilized reading.

Preparation of water samples

A commercial PAHs standard containing the 8 out of 16 US EPA priority PAHs (containing 500 ppm fluorene, phenanthrene, pyrene, benz [a] anthracene, benzo [b]
fluoranthene, Benzo [a] pyrrole, benzo [g,h,i] perylene and indeno [1,2,3-cd] pyrene each) was supplied by Sigma Aldrich, USA and delivered by Bristol Scientific, Lagos. The standard (500 ppm) was run on the GC-MS to calibrate the instrument prior GC-MS quantification.

**Extraction procedure**

Water samples were extracted by liquid-liquid extraction method (Diaz-Moroles et al., 2007) and concentrated. A 150-mL water sample was transferred to a 250-mL separating funnel, and 9 mL of dichloromethane was added. The separating funnel was shaken for a period of 2 min, and the organic layer was separated and collected in a 50-mL flat-bottomed flask containing 2 g of anhydrous sodium sulfate. The extraction step was repeated several times, and the resulting extracts were combined. The combined extract was concentrated to near dryness using rotary evaporator, transferred into amber vial and kept in the refrigerator prior to analyses (Diaz-Moroles et al., 2013).

**GC-MS conditioning**

An Agilent Technologies GC-MS comprises of 7890A gas chromatography and MS 5975C mass spectrometer detector was used in this study. The instrument comprise a column thickness of 0.25 μm and Helium as carrier gas at the rate of 1 mL/min. temperature programme of initial temperature at 60 °C hold for 0.5 mins then ramp to 240 °C at the rate of 10 °C/min to final temperature at 300 °C hold for 6 mins.

**Sample characterization**

The extracts were analyzed using gas chromatography coupled with mass spectroscopy (GC-MS) for both qualitative and quantitative analyses (Christensen et al., 2005; Gomes, et al., 2009).

**Statistical analyses**

One-way analysis of variance (ANOVA) of the resulting PAHs and their maximum allowable concentrations water was carried out using SPSS (Braun, 2012). Correlation coefficient was used to compare concentrations of PAHs in water samples to their respective control samples at α = 0.05 level of significance.

**Method validation**

For the Relative Percent Difference (% RPD); A solvent blank was run prior to analysis. After every sample, a blank was run as duplicate. The Relative Percent Difference (% RPD) was estimated as follows:

\[
\text{Relative Percent Difference (RPD)} = \left( \frac{2(D_1 - D_2)}{D_1 + D_2} \right) \times 100
\]

Where \( D_1 \) is concentration of the analyte in the first duplicate sample and \( D_2 \) is the concentration of the analyte in the duplicate sample (Faggote & Olanipekun, 2013).

For Limit of detection (LOD) and limit of quantification (LOQ); calculations were based on the concentration of the blank sample run.

\[
\text{LOD} = \frac{3s_b}{a}
\]
\[
\text{LOQ} = \frac{10s_b}{a}
\]

Where \( s_b \) stands for the standard deviation of the blanks and \( a \) is the slope of the calibration curve (Fabbri et al., 2013).

**Results and discussion**

**Physicochemical properties of water samples**

Electrical conductivity: Electrical conductivity is an indirect indication of the strength of nutrient solution. Electrical conductivity for the water sample ranged from 9.01 to 14 μScm⁻¹. The electrical conductivity increases as the PAHs concentration decreases in RS to RD in water samples. Conductivity of the water for the whole study area stands at an average of 11.54 μScm⁻¹. This provides an image of very little solute dissolution generally in the river, rapid ion-exchange between the soil and water, or basically a poor and rather insoluble geologic rock and mineral types (Oyem et al., 2014). Temperature: Temperature values of the water sample ranged from 28.63–28.90 °C with an average value of 28.77 °C. The values are within the documented range (26.5-30.1) for similar study (Akerolodu and Simeon, 2010). These suggest that the water temperature is usually ambient and good for users who choose cool to warm water. It is equally good for the specific reason of water quality; since high temperature adversely impact water quality by improving the growth of micro-organisms which may upturn taste, odour, colour and corrosion problems (UNICEF, 2008).

**pH:** Table III reveals that the pH values of the water samples fall within the range of alkaline, hence, most of the nutrients mentioned may be present in the water (Ifelola and Alaba, 2014). The pH values estimated in this study (pH7.57-7.94) also agrees with the upper limit of 7.64 reported by Akerolodu and Simeon (2010). for bitumen wetlands.
Reports on pH from both studies falls within the range (6.5-9.5) set by WHO (1998). Turbidity: The mean turbidity values in this study were above the 5 NTU, which is the accepted limit for drinking water. The high turbidity values in the samples can be attributed to run-off into water bodies. Values above the accepted limit were also reported by Oyakhilome et al., (2012) and Suleiman and Audu, (2014).

**GC-MS quantification**

The chromatograms of water samples were presented in Figures 2-4. Table IV show the PAHs concentrations in water samples collected during dry season (March, 2016). In terms of individual PAH composition, only few PAHs are detected in water samples. The levels of PAHs found in this study ranged from 2.36-14.68 ppb, where benzo [e]pyrene was the most dominant. Abstracted in a similar report is the fact that Benzo[e]pyrene found in water samples from the Piracicaba River and the Doce River in the municipality of Ipatinga, Brazil exceeded the value set by legislation by 80% (da Costa Lima et al., 2015). All the compounds except benzo[e]pyrene in RS and RD and indeno[1,2,3-cd]pyrene in RD are below maximum allowable concentrations in water. tPAHs in the water samples ranged from 14.44 ppb in RS to 19.99 ppb in RD. Several PAHs containing 2-3 aromatic rings have significant acute toxicity to aquatic organisms and those containing 4-6 aromatic rings in their structure, are known to be mutagenic, carcinogenic and inducers of tumors in manuals. Aquatic organisms can accumulate PAHs from water, sediment and food. Unlike the findings from this work,

**Table III. Physicochemical properties of water samples from Irele bitumen seepage**

| Parameter     | RS    | RD    | RC    |
|---------------|-------|-------|-------|
| pH            | 7.94 ± 0.01 | 7.85 ± 0.02 | 7.53 ± 0.02 |
| Temperature (°C) | 28.63 ± 0.25 | 28.90 ± 0.40 | 28.99 ± 0.46 |
| EC (µScm⁻¹)   | 9.01 ± 0.21 | 14.33 ± 0.31 | 12.27 ± 0.30 |
| Turbidity (NTU) | 38.15 ± 0.26 | 41.72 ± 0.72 | 42.18 ± 0.32 |
| Total Organic Carbon (%) | 1.18 ± 0.02 | 1.85 ± 0.06 | 2.11 ± 0.03 |

research by Karlsson & Viklander (2008) unveiled that all measured PAH were found in in Water and Sediment samples in gully pot mixture and approximately 22% of the total 16 PAH were in dissolved phase.
The concentrations of PAHs at various points in water samples were very low or below detection limit indicating small amounts of the pollutants in the river especially in the dry season due to their lipophilic nature. This is in agreement with the results of Teaf (2008) and Fagbote and Olanipekun (2010). Emissions from a host of mechanisms including storm, water runoff, direct deposition, and surface runoff from roadways and discharges from boats contribute heavily to aquatic pollution. Pyrene, a four ring PAH was not detected and quantified in all the water samples. This was also the report of Fagbote and Olanipekun (2010) wherein four out of five sediment samples in bitumen fields were void of pyrenes. Generally, The availability and concentrations of the studied analytes vary across the study area. The high molecular weight (HMW) PAHs are the prevalent with B(e)P present in most of the samples.

**Correlation study**

The linear regression analysis (Figure 5) between some physicochemical parameters and mean concentration of PAHs was carried out in this study. The linear regression analyses for PAHs concentration and total organic carbon indicated low positive correlation in samples ($R^2 = 0.002$). This shows that the sites were specifically contaminated at different levels by cumulative parametric factors. This also means that contamination of water with PAH in the area is from various anthropogenic sources. The most significant sources of PAHs in an environment can be known with the ratios between pairs of concentrations of individual PAHs (Fagbote and Olanipekun, 2013). Other parameters are variously correlated to the mean concentration of PAHs in the samples. All the physicochemical parameters studied in the water samples except pH show positive correlation.

Concentration of PAHs in water samples are significantly correlated to their respective control samples at $\alpha = 0.05$ level (2-tailed) of significance. However, one-way ANOVA of concentrations of PAHs in water samples and their respective maximum allowable concentrations (MAC) in soil and water

**Table IV. PAHs concentration in water samples**

| PAH              | PAHS in Water Sample (ppb) | ΣPAH (ppb) | MAC (ppm) |
|------------------|-----------------------------|------------|-----------|
|                  | RS  | RD  | RC  |           |           |           |
| Fluorene         | BDL | BDL | BDL | BDL       | 3.0       |
| Phenanthrene     | BDL | 4.30| BDL | 4.30      | 3.0       |
| Pyrene           | BDL | BDL | BDL | BDL       | 3.0       |
| Benz[a]anthracene| BDL | BDL | BDL | BDL       | 0.005     |
| Benzo[b]fluoranthene | 2.36 | 3.75 | 2.56 | 5.86      | 0.005     |
| Benzo[e]pyrene   | 12.08 | 14.68 | 1.82 | 26.76     | 0.005     |
| Indeno[1,2,3-cd]pyrene | BDL | 5.62 | BDL | 5.62      | 0.005     |
| Benzo[ghi]perylene | BDL | 5.99 | BDL | 5.99      | 3.0       |
| tPAHs            | 14.44 | 19.66 | 1.72 | 34.10     | 3.0       |

BDL is below detection limit, MACs- Maximum Allowable Concentrations of PAHs in Soil and Water

| PAH        | LOD (ppb) | LOQ (ppb) | % RDP |
|------------|-----------|-----------|-------|
| Fluorene   | 0.03      | 0.10      | 2.90  |
| Phenanthrene | 0.18    | 0.61      | 1.13  |
| Pyrene     | 0.03      | 0.10      | 1.24  |
| Benz [a] anthracene | 0.10   | 0.32      | 5.78  |
| Benzo [b] fluoranthene | 0.12 | 0.40      | 4.40  |
| Benzo[c] pyrene   | 0.12      | 0.40      | 4.49  |
| Indeno [1,2,3-cd] pyrene | 0.08 | 0.25      | 2.30  |
| Benzo [ghi] perylene | 0.06    | 0.21      | 1.13  |

**Table V. LOD, LOQ and % RDP as estimated from the GC-MS analysis**
shows p>0.05. This indicated that the PAHs’ concentrations detected are not significant and the water is safe from the PAHs studied when compared with PAHs MAC in water.

**Method validation**

**Conclusion**

Highlights from this studies showed that water samples from the studied source (RS) and delivery (RD) points were contaminated with PAHs with concentrations below the maximum allowable concentrations except for few 4-rings members. Difference in concentration of PAHs in water samples and their respective control samples are significant (p>0.05). The overall concentration of the studied PAHs poses no health threat on the environment and man.

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