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Occurrence and Dynamics of Hydrocarbon in Periwinkles *Littorina littorea*

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Abstract

The cumulative effects of small accidental spills in the marine environment can have significant impacts on marine wildlife. Aquatic fauna are often examined for evidence of hydrocarbon exposure, as it may pose direct threat to their health and consequently humans. The composition and spatial distribution of hydrocarbons especially polycyclic aromatic hydrocarbons (PAHs) were investigated in the tissue of periwinkles *Littorina littorea* from two carefully selected locations within the oil-polluted coastal area of Ondo State, Nigeria using gas chromatography with flame ionization detector and mass spectrometry (GC/FID/MS). The tissues were sampled and analyzed to evaluate the spatial extent and persistence of pollution, as well as their potential for adverse effects. Complex series of compounds, many of which were considered biotransformed products were obtained. The PAHs ranged; not detectable (nd) – 56.23 µg/g dry weight (dw) and nd - 79.43 µg/g dw during the dry and wet seasons respectively. The total PAHs (∑PAHs) during the wet season was almost 100% greater than ∑PAHs during the dry season; a factor attributed to run-off from contaminated land during the wet season. The levels of PAHs obtained were partly attributed to the lack of aryl hydrocarbon hydrogenase (AHH) system in *L. littorea.* Relative ratios of low molecular weights, LPAHs (<3-ring) compounds to those with high molecular weights, HPAHs (>4-rings) suggested a petroleum related origin for the PAHs detected in the organism. The PAH and bioaccumulation factor (BAF) are significantly correlated ($P<0.05$, $r= 0.908$). The levels of PAHs in the tissues were relatively low compared to world-wide locations reported to be chronically contaminated by oil.

Keywords: Oil spill, hydrocarbons, gas chromatography; mass spectrometry; periwinkle.

Deniz Salyangozu *Littorina littorea*'da Hidrokarbonun Varlığı ve Dinamikleri

Özet

Sucul ortamda meydana gelen küçük kazaların kümülatif etkileri, suda yaşayan canlıların doğal yaşantısında önemli sonuçlara yol açabilir. Akuatik fauna sık sık hidrokarbona maruz kalıp kalmadığıyla ilgili incelenir. Çünkü akuatik faunada hidrokarbonun bulunması direkt olarak içinde yaşayan canlılara ve dolaylı olarak da insanlara tehdit teşkil eder. Nijerya'nın Ondo Eyaletindeki petrol kirliliği bilinen alanlarda ve özenle seçilen iki kıyısal bölgede deniz salyangozu *Littorina littorea*'nın dokusunda hidrokarbonun içerik ve uzamsal dağılımı özellikle de polisiklik aromatik hidrokarbonların (PAHs) varlığı, alev iyonlaştırıcı detektörlü ve kütle spektrometreli gaz kromotografisi (GC/FID/MS) ile araştırılmıştır. Kirliliğin sürekliliğini ve mekansal boyutunu hem de yan etkilerini değerlendirmek için doku örneklemesi ve analizler yapılmıştır. Bir çoğunun biyodönüşüm ürünleri olduğu düşünülen kompleks seriler halinde bileşikler elde edilmiştir. PAHs dağılımı: sırasıyla kuru ve yaş sezonda ölçülemez (nd) -56,23 µg/g kuru ağırlık (dw) ve nd -79,43 µg/g dw'dir. Yaş sezon boyunca toplam PAHs (∑PAHs), kuru sezondaki toplam PAHs'den (∑PAHs) %100 daha büyük çıkmıştır; çünkü yaş sezon boyunca kontamine bölgeden kaçış gerçekleşmiştir. Elde edilen PAHs seviyeleri kısmen *L. littorea*'da aril hidrokarbon hidrojenaz sisteminin yokluğuna bağlanabilir. Düşük moleküler ağırlıklı LPAHs (<3-halka) bileşiklerin, yüksek moleküler ağırlıklı HPAHs (>4-halka) bileşikler ile göreli oranı, incelenen organizmadaki PAHs'lerin petrol kaynaklı olduğunu akla getirmektedir. PAH ve biyo birikim faktörü (BAF) önemli derece ($P<0,05$; $r= 0,908$) korelasyon göstermektedir. Tüm dünyadaki kronik petrol kontaminasyonu olduğu rapor edilen diğer bölgeler ile kıyaslandığında dokulardaki PAHs seviyelerinin nispeten düşük olduğu bilinmektedir.

Anahtar Kelimeler: Petrol bulaşması, hidrokarbonlar, gaz kromotografisi; kütle spektrofotometresi; deniz salyangozu.

Introduction

Crude oil is a complex mixture of many chemical compounds, composed primarily of phenol and aromatic hydrocarbons including polycyclic aromatic hydrocarbons (PAHs) many of which are of toxicological interest (King, 1988; MacFarland, 1988; Cram *et al*., 2004). The PAHs can originate from both anthropogenic sources (such as coal gasification, accidental oil spills, as well as natural processes such as fossil fuel and wood combustion) and biogenic sources (with biogenic precursors like terpenes, pigments, and steroids) into the environment (Gevao, 1998; Asikainen *et al*., 2002; Law *et al*., 2002; Koh *et al*., 2004; Takasuga *et al*., 2007; Ololade and Lajide, 2009). As a result of their hydrophobicity and low water solubility, and vapour pressures, occurrence of PAHs in the environment is of concern due to their carcinogenic properties, and their ability to exert toxic effects through the aryl hydrocarbon receptor (AhR) mediated mechanism, similar to those of dioxins (Ghauch *et al*., 2000; Villeneuve *et al*., 2002; Ikenaka *et al*., 2005; Diane *et al*., 2006). A lot of critical reviews on hydrocarbon measurement and biotransformation in biological tissues for health effect monitoring and foodstuffs for safety reasons have been reported (Stroomberg *et al*., 2004; Shi *et al*., 2006). The aromatic hydrocarbons showed high solubility and concentration in blood and low concentration in brain, liver and kidney and these have a tendency to accumulate in adipose tissues with high probability for biotransformation (Park and Holiday, 1999; Jocelyne, 2004). It also results in death of wildlife surrounding oil spills including fish, periwinkles, seabirds and marine mammals (Moritam *et al*., 1999; Carrasco *et al*., 2006).

Apart from direct absorption from the water when oil spills, marine species such as periwinkles; a bottom feeder, are at greater risk due to close contact to the sediments (Karageorgis *et al*., 2005; Emira and Mirjana, 2007). However, these species that inhabit the intertidal zone possess a high degree of metabolic plasticity that enables them to tolerate the constantly changing environmental conditions imposed by the tidal cycle. One of such species, is the free living epibenthic animal, the periwinkle snail *L. littorea.* This species is highly tolerant of oxygen deprivation and has also developed the ability to survive freezing. *Littorina littorea* along with some marine organisms like the periwinkles (*P. aurita*), mangrove oysters (*C. gasar*) and mussels (*M. edulis*) are preferred pollution biomonitors because apart from being sedentary or bottom feeders, they are good accumulators of heavy metals and PAHs (Rainbow and White, 1989; Wilson *et al*., 1992). The ability to assess bioaccumulation in periwinkle is important for understanding food-web dynamics and transfer of contaminants throughout the ecosystem. This concept of bioconcentration/bioaccumulation factor (BCF/BAF) is important in evaluating contaminant toxicity. In aquatic toxicology, it is important to determine the nature and proportion of the total toxicant available to an organism to establish the degree to which bioaccumulation and adverse effects of hydrocarbons can occur. This can be achieved by using sophisticated equipment like the hyphenated technique of gas chromatography mass spectrometry (GC-MS). This hyphenated technique has been used by several authors in the quantification and qualitative evaluation of hydrocarbons components in biota (Stroomberg *et al*., 2004; Jack *et al*., 2005; Poster *et al*., 2006; Perugini *et al*., 2007).

Aquatic assessment using the shellfish *L. littorea* is rare. The present study is one major attempt to understand what the spatial and temporal impacts of hydrocarbon spills on the gastropod periwinkle *L. littorea* collected from Ondo oil-producing coastal area in Western Nigeria, may look like. Considerable amounts of periwinkles are obtained daily for food. *Littorina littorea* is a commercially valuable periwinkle in the Niger Delta; a zone to which the study area belongs. Their value compares favourably with those of domestic livestock and fish (Dambo, 1993). In fact, the best soup within the Niger Delta region "Idikhaikon" is made with *L. littorea* and some other species. It is therefore important to investigate the ability of this species as a bio-indicator of oil polltion.

Materials and Methods

Study Area

Figure 1 shows the study area from which samples of periwinkles *L. littorea* were randomly collected. Ondo State is one of the oil producing state, located in the south western part of Nigeria. The climate is tropical with two distinct seasons: the rainy season (April–October) and dry season (November–March). The water temperature and salinity during the dry season (wet season in brackets) ranged from 29.1–31.8°C (27.3–28.1°C) and 4.32–4.36 (3.89–3.92), respectively. The sampling sites are locations that are previously noted for large biological production and especially important for commercial fish and periwinkle. Reduction in economic productivity started in 1996 when the first oil spill occurred (ODSEPA, 2000). Oil spills of various sizes occur periodically in the area. Have been on large scale or have occurred in circumstances resulting in limitless damage to the marine environment. Large hectares of mangroves ecosystem have been destroyed through toxicity from persistent oil spillage. The yearly economic loss due to oil spill by the inhabitants cannot be quantified. Consequent upon the negative impacts of this unpalatable experience and previous studies on the sediment (Ololade and Lajide, 2009; Ololade 2010), this investigation was carried out in order to provide information on the impacts oil spill on the tissues of common edible *L. littorea* and

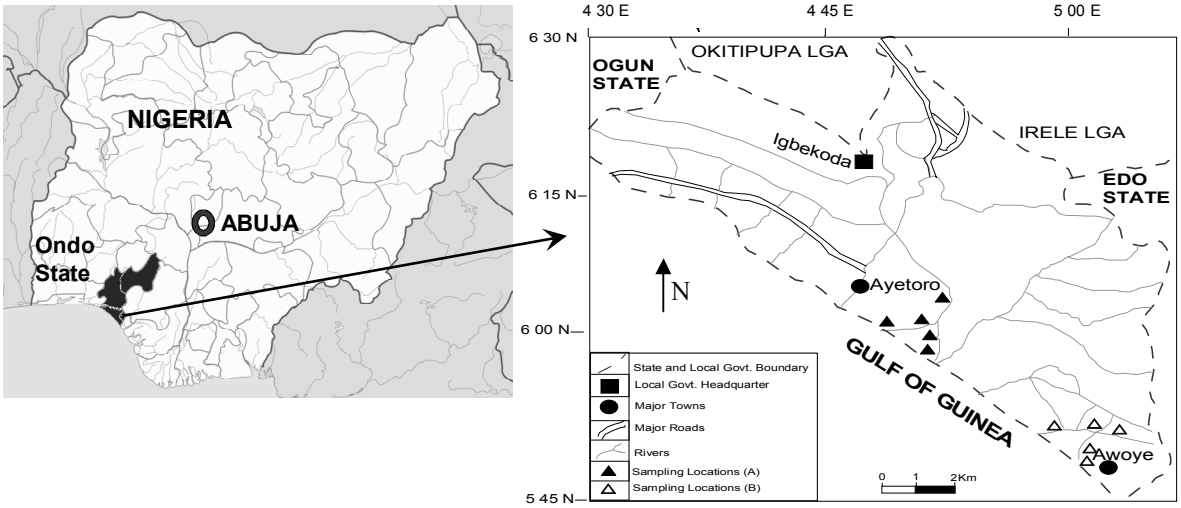

Figure 1. Map of the study area (inserted is the area map of Nigeria and Africa showing the geographical location).

possibly, the impact of feeding nature on pollutants concentration of the bottom feeding periwinkle.

Test Organism/Sampling

The gastropod *L. littorea* is free living epibenthic animal found in intertidal locations and is widely distributed in coastal and estuarine areas in Ondo State. Basically, two locations (Site-A and Site-B as indicated in Figure 1) were selected within the study area. Some of the variables that influenced sites selected include, proximity to oil wells locations, high population density and high level of socio-economic activities, particularly fishing within the area. Dry season samples were collected in November 2004 while the wet season samples were obtained in June 2005. This was to enable investigation of possible seasonal differences in uptake/release of oil pollutants. Periwinkles *L. littorea,* numbering about thirty (30) were picked randomly from their various habitats along the river bank/intertidal zone (sediments) during each season. Samples were thoroughly washed for any adhered sediment particles. Efforts were ensured to pick larger periwinkles during the wet season as compared to the dry season. This was deliberate in order to assess the impact of bioaccumulation within the period under study since growth was expected to have occurred before the wet season collection. The periwinkles were wrapped in hexane-rinsed aluminium foil, labeled, placed on ice in the field and stored at -20°C upon return to the laboratory.

Sample Preparation and Clean-up Procedure

Extraction of hydrocarbons and clean-up of tissues for gas chromatography analysis was based on literature guidance (Ashok *et al*., 2004). The soft part of the periwinkles was obtained by cracking the shells. Approximately 10 g were placed into a clean mortar, then ground with pestle with 40 g of anhydrous sodium sulphate. The samples were extracted in soxhlet apparatus with three aliquots of methylene chloride. Internal standard (1 ml tetracosane for the aliphatic and 1 ml anthracene for the aromatic were used). The solvent extract was separated from the tissues using a separatory funnel, and then dried by mixing it with sodium sulphate. The extracts were eluted through a silica-alumina glass column for the removal of polar lipids and other biogenic interferences, and the samples were concentrated to a volume of 5 ml. The fractions were combined and dissolved in a known amount of dichloromethane prior to GC measurement. For quality assurance, a method blank was analysed.

Gas Chromatography-Mass Spectrometric (GC-MS) Analyses

The capillary GC-MS analysis were performed on a Hewlett-Packed (HP) 6890 GC series instrument coupled with a 5975 Hewlett-Packed mass spectrometer (MS). The system control and the data acquisition system were controlled by a MS-DOS compatible work station. One microlitre (1 µl) of the worked up sample was injected into the GC instrument using a 10 µl syringe size. The gas chromatograph was equipped with a split injector (purge delay of 15 seconds, purge flow of 6.8 ml/min; injection temperature 250°C and pressure of 2.3 kpa). The capillary column used was of the Agilent 1909IS-433 models with dimensions of 30 m x 0.25 mm ID x 0.25 µm film thickness. Helium was used as a carrier gas with initial flow rate of 0.7 ml/min and average velocity of 30 cm/sec, the column was kept at 40°C initially, and then programmed to 325°C. The mass

spectrometer was operated from 35 to 500 Dalton (SCAN mode). The peaks in the chromatogram were identified by comparison of the retention times and spectra of reference compounds with those in the sample. The peaks were quantified using the flame ionization detector (FID).

Statistical Analysis

We performed statistical analysis by combining data from the two seasons. Statistical differences in seasonal PAHs level were determined by one-way ANOVA, followed by Duncan new multiple range test. Relationships between PAHs and other controlling factors were determined by bivariate correlation using the Pearson coefficient in a two-tailed test ($P<0.05$). All analyses were performed using SPSS software (version 13.0).

Results

The gas chromatograms of the hydrocarbons in the tissue of *L. littorea* from site-A and site-B in both dry (November) and wet (June) seasons are presented in Figures 2a-2d respectively. The gas chromatogram gave a total of 35 and 46 identifiable peaks for both site-B and site-A during the dry and wet seasons respectively. Table 1 presents the concentration of PAHs in *L. littorea* at the two sites for both wet and dry seasons. At site-B, phenanthrene has the highest concentration was 49.24 µg/g during the dry season and 73.9 µg/g in the wet season. Similar trend in phenanthrene was also observed at site-A for both seasons. Anthracene was second highest in

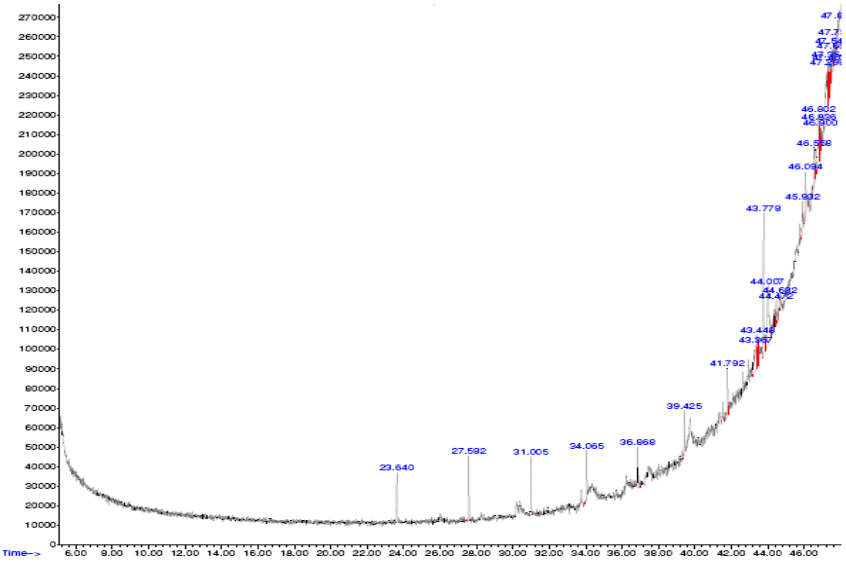

Figure 2a. Gas chromatograms of hydrocarbons in periwinkles (*L. littorea*) from site-B during the dry season.

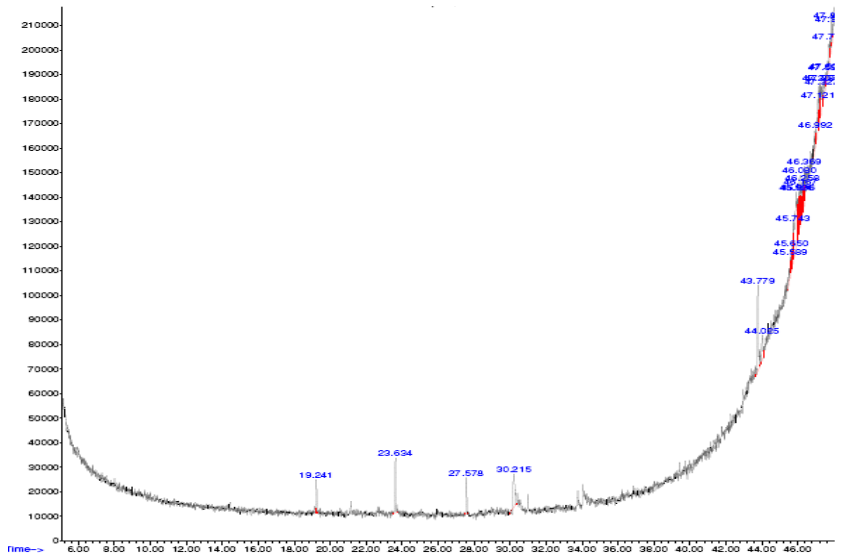

Figure 2b. Gas chromatograms of hydrocarbons in periwinkles (*L. littorea*) from site-B during the wet season.

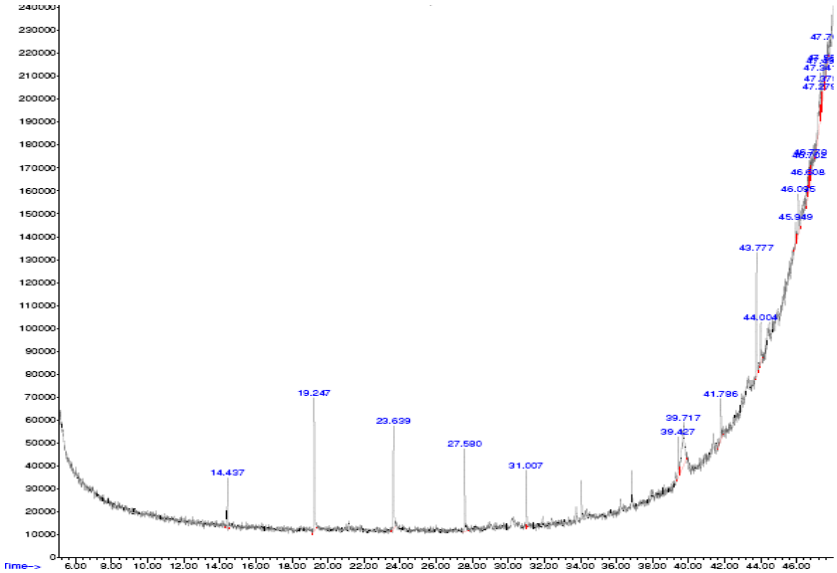

Figure 2c. Gas chromatograms of hydrocarbons in periwinkle (*L. littorea*) from site-A during the dry season.

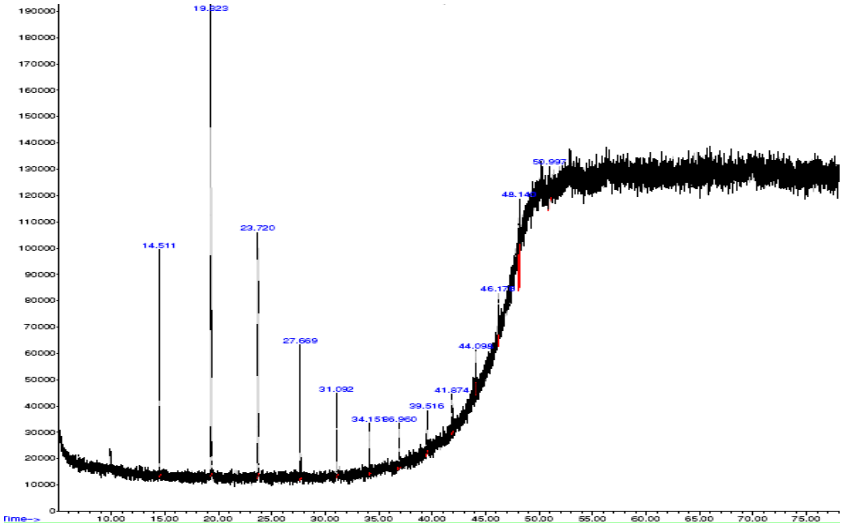

Figure 2d. Gas chromatograms of hydrocarbons in periwinkle (*L. littorea*) from site-A during the wet season.

Table 1. The mean concentration (± s.d) of PAHs in *Littorina littorea* (µg/g dw)

Sampling Locations	Site A-D†	Site A- W‡	Site B-D†	Site B-W‡
Anthracene	25.02±3.46	47.24±4.92	32.10±5.91	44.21±6.72
Phenanthrene	49.24±5.03	73.91± 9.05	56.23±8.24	79.43±9.47
Azuleno(2,1-b)thiophene	4.28±0.45	4.32±1.02	2.04±0.01	2.15±0.08
Dibenzothiophene	0.09±0.02	0.04±0.02	nd	nd
Benz[a]anthracene	9.22±2.47	15.04±4.84	12.01±3.09	17.08±3.21
Dibenzo[a,h]anthracene	7.04±1.24	12.95±2.07	10.74±3.41	19.22±3.07
Carbazole	nd	16.24±4.67	nd	13.21±2.06
Benz(e)azulene	0.56±0.31	1.09±0.23	3.02±1.03	5.41±1.54
Dibenzo(b,e)(1,4)diazepine	nd	nd	0.52±0.03	2.01±0.04
Benzo(1,3)chromene	nd	6.73±2.07	nd	9.94±2.25
Pyrolo(3,2-b)benzofuran	2.49±0.42	4.06±0.93	2.78±0.07	5.18±1.34
Ethylacridine	1.78±0.33	nd	0.54±0.01	2.76±0.71
Other PAHs	14.42±8.49	23.45±9.34	19.45±8.79	29.76±8.98
SumPAHs#	114.14±16.23	205.07±23.88	139.43±18.95	230.36±23.69

† and ‡ represent respectively small (3.0–3.7 cm in length) and large (4.2–4.9 cm in length);
s.d: standard deviation; #: s.d calculated without using other PAHs. nd: not detectable; dw: dry weight; D – dry season, W – wet season.

concentration and followed similar trend. Carbazole was not detected during the dry season at both sampling sites. A concentration of 16.24 µg/g at site-B and 13.21 µg/g at site-A was found in the wet season. Dibenzo(b,e)(1,4)diazepine was not detected at all in site-B but was found at very low concentration in site-A. The sum total of the PAHs in the tissue extract of *L. littorea* was lower in the dry season than in the wet season. Site-B had ∑PAH 114.14±16.23 µg/g (dry season) and 205.07±23.88 µg/g (wet) while site-A has the highest 139.43±18.95 µg/g (dry season) to 230.36±23.69 µg/g (wet season). Table 2 presents the identities of other compounds isolated from the tissue extracts of *L. littorea*. The identity of each compound was based on the retention times and comparison of their mass spectra data in the GC- mass spectrometer computer library. These categories of compounds with the exception of a few are functionalized hydrocarbons. The mass spectrometric details (those of toxicological importance) of some identified compounds are presented in Figure 3.

Discussion

The peaks of the chromatograms followed similar trend except for the wet season in extracts from site-A where several unresolved complex mixture were noticed with completely different patterns of retention time of identified compounds. The unresolved complex mixture may be responsible for the limited number of identified compounds from this extract. The unresolved nature of the mixtures coupled with some identified heterocyclic compounds is indicative of petroleum hydrocarbon source (Genki, 1982). The ∑PAH found in the tissue of *L. littorea* is a cause for concern because this species constitutes a major part of food to the people of the area studied and a world-wide food source. The source of these hydrocarbons can only come from frequent oil spills that occur in the areas under study.

Contaminated sediments can directly affect bottom-dwelling organisms and represent a continuing source for toxic substances in aquatic environments that may affect wildlife and humans via the food chain. This was found to be true in the present study because greater number of PAHs were detected in the tissues of *L. littorea* (Table 1), than in sediments as reported in another study (Ololade and Lajide, 2009). The tables equally reflect on the strength of *L. littorea* as good indicator of hydrocarbon pollution. Overall, the contamination pattern for total PAHs within the two sites was observed in the following decreasing order: Site-B > Site-A at both seasons**.** Anthracene and phenanthrene were not only predominant but also abundant within the two sites at both seasons; azuleno [2,1-b]thiophene; pyrrole [3,2-b]benzofuran and ethylacridine were detected at quantifiable concentrations at both sites except for the absence of ethylacridine during the wet season at Site-A; carbazole and benzo [1,3]chrome were both detected only during the wet season but at abundant concentration at both sites; other PAHs (many occurring in modified forms) were detected at variance at both sites during the two seasons. The presence of some modified forms of PAHs (with their RTs in brackets) such as; 9,10-diethyl-9-10-dihydroanthracene (44.000); 5-methyl-5H-Naptho[2,3-c] carbazole (23.637); 2-benzo[1,3]dioxo-5-yl-8-methoxyl-3-nitro-2H-chrome (23.721); 9,10-dihydro-9,9,10-trimethylanthracene (34.092) and 7-methyl-7H-dibenzo(b,g)carbazole (46.083) were also observed. Consequently, consumption of contaminated periwinkle may lead to human exposure to these complex series of compounds. Positive correlations ($P<0.05$) were observed between PAHs that were detected at both locations and between the

Table 2. Other compounds identified with their retention index

Some Other Compounds	RT/min	Area (%)	m/z (% Quality)
3-methyl heneicosane	44.654	2.55	43.1(70.1),57(78.5),281.2(100)
11-methyl nonacosane	45.928	2.96	41.0(76.2),97.0(79.4),57.1(81.4)
13-methylethyl-cyclopentane	46.560	4.42	97.1(48.),83.0(57.6),69.1(100)
Bis (2-ethylhexyl) phthalate	43.782	11.09	167(34),57(35),149(100)
11H-Dibenzo(b,e)(1,4)diazepine-11-one	47.534	2.11	195(79),281(86),238(100)
9,10-Anthracenedione	50.999	15.64	152(60),180(82),208(100)
4,5-Ethylene-8,9-dimethoxy-6-phenanthridone	47.389	1.24	119(36),139(39),281(100)
Benzofuran-2-one	46.259	1.47	57(49),73(92),207(100)
1,2-benzendicarboxylic acid	43.772	11.09	167(34),37(35),149(100)
5-methyl-2,2,2-trimethyl benzoic acid	39.430	8.84	147(58),281(87),73(100)
1,3-bis (trimethylsilyl) benzene	39.430	3.26	207(66),73(83),40(100)
Dodecamethyl cylopentasiloxane	14.511	3.64	267(73),73(92),355(100)
Dodecamethyl cyclohexasiloxane	19.243	3.38	429(52),73(66)341(100)
Dodecamethyl pentasiloxane	23.637	2.79	147(54),281(57),73(100)
Cholesta -3,5-dien-7-one	39.720	11.61	55(63),174(76),40(100)
Phosphetane	46.840	0.13	282(34),43(36),91(100)
3-ethylcyclopentanecarboxamide	43.995	3.16	73(34),45(53), 207(100)
2-methyl-1-adamantaneacetamide	47.337	5.59	41(49),73(67),207(100)

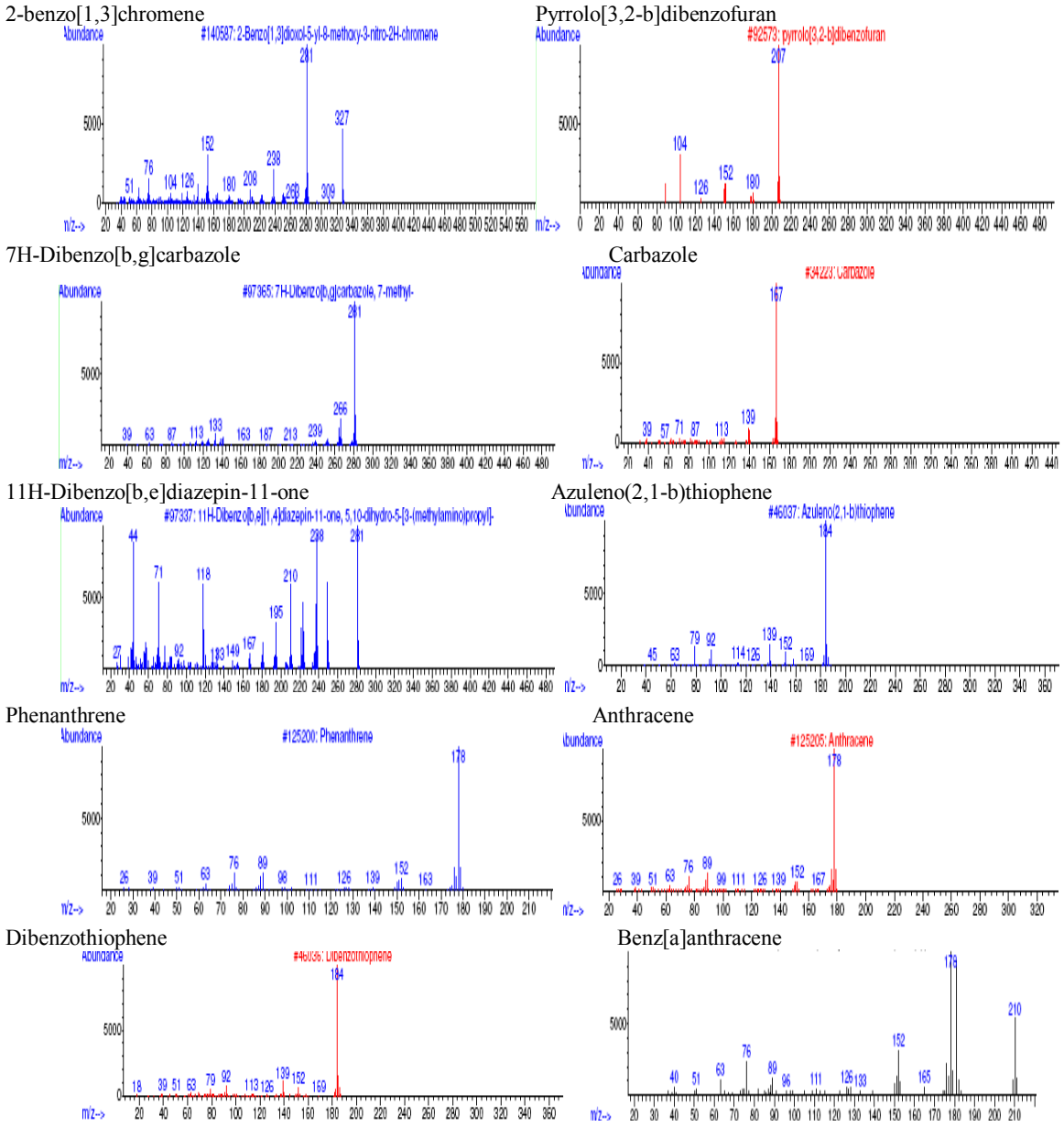

Figure 3. Mass spectrophotometric details of some compounds of toxicological interest.

two sites (Figures 4a and 4d). All these are indicative of probable similar source and distribution pattern. Using the result of the GC analysis of the water as reported (Ololade, 2008), PAH-BAF in *L. littorea* (Figure 5) was also significantly positively correlated ($r = 0.908$, $P<0.05$).

Diagnostic Assessment of Possible Elevated Concentrations and its Significance

Lower molecular PAHs (LPAH) (<3 rings) were very few as can be observed in Table 1. They constituted about 25% and 30% of the total amount of PAHs determined in the study during the dry and wet seasons respectively. The contribution of HPAHs (4 and 5 rings) was observed to be dominant across the study sites. The methylated phenanthrenes/anthracene and the parental PAH phenanthrene, can be contributed by direct spillage of petroleum compounds. This hydrocarbon pattern, mainly dominated by the heavier hydrocarbons, alkyl-substituted PAHs suggests exposure to highly weathered petroleum mixture (Neff, 2002; Barron and Holder, 2003).

The enzyme, aryl hydrocarbon hydroxylase (AHH) is responsible for the metabolic modification of foreign organic compound in some biota. This has been reported to be lacking in *L. littorea* (Neff, 1979) and may be responsible for the high level of PAHs reported in this study. In addition, the gastropods; the class to which *L. littorea* belongs has been found to exhibit reduced activity on exposure to sub-lethal concentration of PAHs. This has led to a range of detrimental physiological responses resulting in

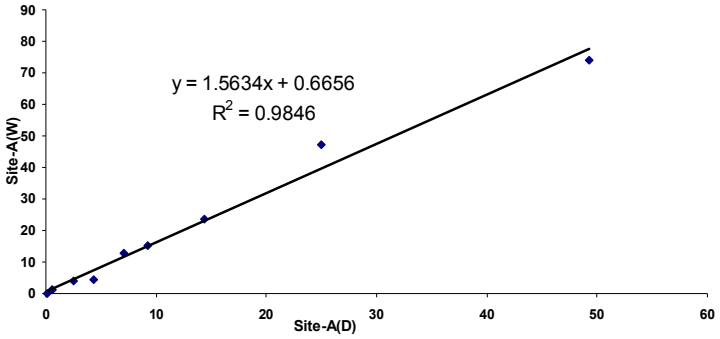

Figure 4a. Correlations of PAHs between dry (D) and wet (W) seasons in site-A.

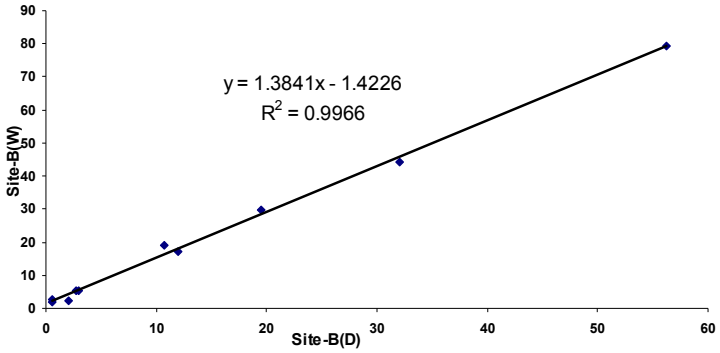

Figure 4b. Correlations of PAHs between dry (D) and wet (W) seasons in Site-B.

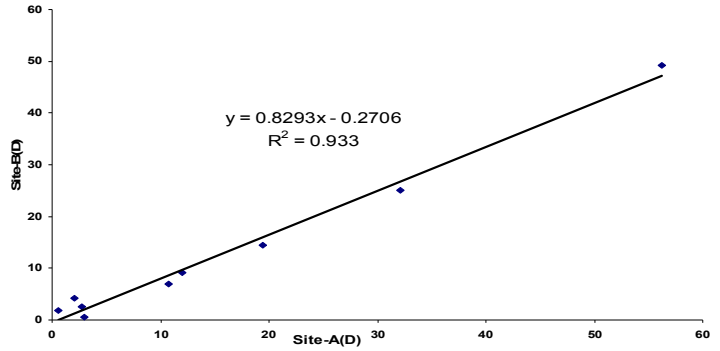

Figure 4c. Correlations of PAHs between the dry (D) seasons at both sites.

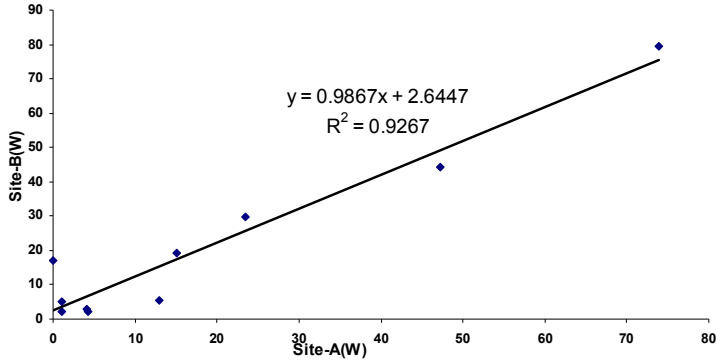

Figure 4d. Correlations of PAHs between wet (W) seasons at both sites.

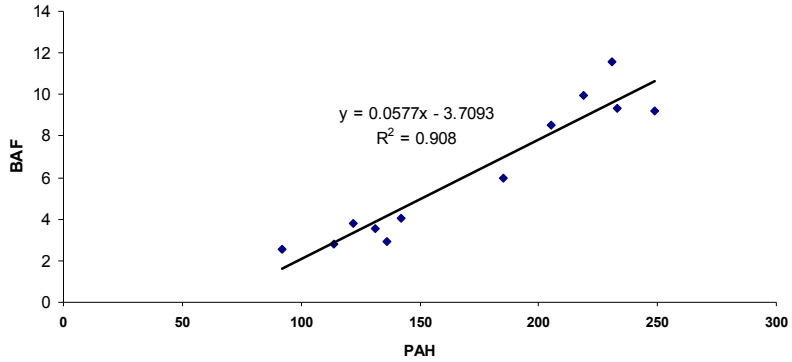

Figure 5. Correlation of PAHs and their bioaccumulation factor (BAF) in the tissues of *L. littorea*. Periwinkles appeared to retain the KPAHs to a greater extent than fish. This is in agreement with literature (Eisler 1987).

histopathological effects such as changes in feeding or other behaviour, growth reduction and reduced reproductive capacity and most importantly, carcinogenicity (Chapman *et al*., 1998; Robertson, 1998; Johana, 2005). Thus, metabolic rates increased due to hydrocarbon association in the body tissues with resultant increase in secretion and excretion. Consequently, energy expenditures increased while less energy (reduced carbon flux) is available for growth and reproduction (Widdows *et al*., 1982; Bayne *et al*., 1982). In mesocosm experiments, hydrocarbon oil has been reported to affect negatively the recruitment of the edible *L. littorea* and its crawling rates (Hargrave and Newcombe, 1973; Linden, 1977; Gray, 1987). All the above effects are particularly of interest in relation to the utilization of this marine resource for food. The low concentration of hydrocarbon in the tissues as compared with locations reported to be chronically contaminated may lead to increased rate of oxygen consumption at the initial stage (Miller and Connell, 1980; Bayne *et al*., 1982).

Variation in PAHs Between Sites

Strong variations in water level, notable stress caused by wave action and reduction in salinity are prominent characteristics of the intertidal zone which may affect concentrations of pollutants in marine species. The analysis of variance (ANOVA, $P<0.05$) shows significant mean difference in concentrations of PAHs between the two sites during the dry season ($p = 0.024$) but without any significant difference during the wet season ($p = 0.093$) probably due to the flow actions of the river system during the wet season. Between the dry and wet seasons at the same location, significant mean differences were recorded; site-A ($p = 0.037$) and site-B ($p = 0.019$). With due considerations to PAHs that were isolated at both seasons, significant positive correlations ($P<0.01$) were recorded between site-A ($r = 0.992$, $N = 8$) and site-B ($r = 0.999$, $N = 9$). Similarly, positive correlations were found between same compounds in dry season ($r = 0.994$, $N = 7$) and wet season ($r = 0.992$, $N = 7$). Slight variations in PAH concentrations between sites suggests different sources of contamination. Different salinity gradients and other environmental factors may result in marked changes in community structure over short distances. However, PAHs originate mainly from petrogenic source (Neff, 2002; Barron and Holder, 2003). Generally lower level of PAHs in the small *L. littorea* during the dry season would be taken as an indicator of higher degree of accumulation in bigger *L. littorea*. The concentration levels are comparable to heavily polluted sites in urban, coastal major oil-producing areas of Rivers and Bayelsa States in Nigeria (Essien and Antai, 2005; Ideriah *et al*., 2005; Jack *et al*., 2005). Moreover, significant proportions of PAHs in the tissues are heavier PAH compounds, which are generally considered to be more carcinogenic. Thus, in terms of PAH pollution, marine seafood's quality of the studied areas may be worse than those of other locations with similar concentration level.

Tissue Tolerance and its Significance

The tissues of periwinkles appear as a suitable indicator of PAHs based on the relatively high tolerance (up to ppm level) to these organic pollutants. The observed values for some of the PAHs were much higher than the 10 mg/l recommended for refinery effluents in Nigeria (Federal Environmental Protection Agency, FEPA, 1991). This further demonstrates the bioaccumulative potential of *L. littorea*. The present levels of PAHs, (all in ppm level) seem not able to pose significant stress to the local benthic *L. littorea*. The hard shell of this organism may probably be playing significant role in exchange of organic pollutants. This assertion deserves further investigation. However, as other benthic organisms may not be as tolerable as periwinkle, it is less certain whether the present levels of these trace organic pollutants are threatening other local benthic organisms.

Conclusion

The present study shows that tissues of *L. littorea* contain high levels of PAHs with high percentage occurring in various modified forms. This is considered a serious signal mainly to resident bottom feeders and to some extent migratory surface feeders. The levels obtained have the potential to cause detrimental histopathological changes to *L. littorea.* Despite the knowledge that had been obtained on the background levels of anthropogenic contaminants in the oil-producing coastal area of Western Nigeria, a number of important links to the establishment of a sound and reliable risk assessment for the entire coastal ecosystem are still not available. The following is a list of recommendations to the authority on works and studies that are necessary in order to assess the risk faced by the coastal ecosystem as a whole as well as to ensure proper conservation of the entire coastal region:

a) Investigation of the loadings and forms of trace organic contaminants (petroleum hydrocarbons including PAHs, total PCBs and organochlorine pesticides) in tissues of economic marine organisms, especially those that are food sources.

b) Assessment of the ecotoxicological effects of trace organic contaminants both on common resident and non-resident bottom/surface feeders.

c) Identification of the sources of trace organic contaminants in the ecosystem.

d) It will also be worthwhile studying the possible sub-lethal effects of petroleum in laboratory set-up.

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