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Research Article
Seasonal Variation of Polycyclic Aromatic Hydrocarbon (PAH) Contamination in *Crassostrea tulipa* (Oysters) and Sediments in Three Ghanaian Coastal Ecosystems

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Abstract

Background and Objective: Polycyclic aromatic hydrocarbons have received considerable attention as environmental organic pollutant in many continents such as Africa, Europe and Asia. Many polycyclic aromatic hydrocarbon compounds have been identified and quantified in virtually all segment of the environment due to their carcinogenicity, mutagenicity and cytotoxicity at very low concentrations. The objective of study was to look at the seasonal levels of polycyclic aromatic hydrocarbons in sediments and *Crassostrea tulipa* (oysters) (bio-indicator) in three water bodies from three coastal ecosystems in Ghana and also assessed the risk involved in their exposure. **Materials and Methods:** Two hundred and seventy oysters and eighty four sediments samples were taken for the two seasons (dry and wet season) from three coastal water bodies at Narkwa, Ada and Anyanui and extracted simultaneously by solvent-solid and Soxhlet extraction. The extracts were analyzed for sixteen polycyclic aromatic hydrocarbons using the Agilent 6890N GC-FID/MS. One and 2-way ANOVA and SPSS were employed for the data and statistical analysis. **Results:** The mean total polycyclic aromatic hydrocarbons levels in oysters from these sites ranged from 66.85-168.59 and 226.24-359.97 μg kg^{-1} in the dry and wet seasons, respectively. Elevated carcinogenic and mutagenic risks (> unit risk of 1×10^{-5}) were associated with the ingestion of oysters from these sites especially for the wet season. The mean total polycyclic aromatic hydrocarbons concentrations in sediments from the three sites also ranged from 78.82-108 and 72.35-136.35 μg kg^{-1} for the dry and wet seasons, respectively. ANOVA conducted at 95% CL showed no statistical significant difference between the sites ($p = 0.905$) and also between seasons ($p = 0.112$) for polycyclic aromatic hydrocarbons levels in oysters. Also, polycyclic aromatic hydrocarbons in sediments were statistically found to be seasonal dependent ($p = 0.007$) but not site dependent ($p = 0.078$). The water bodies from the sites of sampling were polluted since the oysters used as bio-indicators recorded elevated polycyclic aromatic hydrocarbons levels. **Conclusion:** Patrons are advised to minimize their consumptions of oysters and exposure to contaminated sediments from these sites in order to minimize the health effect associated with environmental polycyclic aromatic hydrocarbons.

Key words: PAHs, toxicity equivalency, bio-indicators, sediments, oysters

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are formed as products of incomplete combustion of fossil fuels and other organic matter and major sources include emissions from wood and coal burning, motor vehicles, power stations and refuse incinerators. The PAHs are derive mainly from anthropogenic sources and were widely distributed in the environment, particularly around industrial and urban centers[1]. They are well known class of ubiquitous ecotoxicant that was harmful to human health. Some of them are known to be carcinogenic, mutagenic and teratogenic[2-4]. The recent monograph of International Agency on research into cancer[5] has reclassified the following U.S EPA priority PAH according to their carcinogenicity: Benzo[a]pyrene as definite carcinogen (group 1) dibenz [a, h] anthracene as probable carcinogen (group 2A), whereas naphthalene, benz[a]anthracene, chrysene, benz[b]fluoranthene, benz[k]fluoranthene, benz[j] fluoranthene, indeno (1,2,3-c, d) pyrene were classified as possible human carcinogens[5,6].

Polycyclic aromatic hydrocarbons are known to be accumulated by fish and shellfish and particularly by bivalve molluscs[1]. Oysters were filter feeding biological species found in the marine environment and due to their mode of feeding accumulate a lot of pollutants including PAHs deposited in the marine environment[7]. Thus oysters were usually used as bio-indicators in monitoring pollutions in marine ecosystems.

Ghanaians, especially those along the coast of Volta and Greater Accra regions eat oysters in both the raw as well as the cooked forms, making possible PAH ingestion as a major exposure route for humans. Dermal contact is also increasingly being taken into account as a route of exposure to environmental PAHs as well as inhalation being the primary route of exposure[8].

The cancer control division of Ghana health service (GHS) on February 4, 2011 estimated that 16,600 cases of cancer occur annually in Ghana with an occurrence rate of about 109.5 cases/100,000 persons. The report stated that most of the cases seen in Ghana and other West African countries identified the disease with younger people which were directly opposite to what has been reported in the developed world[9,10]. As a result of the recent monograph[5] which has classified several PAHs as carcinogenic, it is important to investigate the possible risk factors to reduce this increasing cancer cases in Ghana.

Currently, there is no known standard for monitoring environmental pollutants like PAHs in Ghana despite the efforts made by international communities in this regard. This study therefore, sought to monitor the extent of PAH pollution along some coastal water bodies of Ghana using oysters (as bio-indicator) and sediment samples. The study also calculated the risk (incremental risk) involved in ingestion of PAHs in oysters. This study may help to provide standards for monitoring PAHs in Ghana and thus may help to reduce significantly the devastating upsurge of cancer cases in Ghana and the world as whole since PAHs are well-known carcinogens.

MATERIALS AND METHODS

Sample collection: *Crassostrea tulipa* (oyster) and sediment samples were taken from three sites during the month of June, 2010 -September, 2010 for the wet season and October, 2010-January, 2011 for the dry season. Sample collection was done from a river at Ada, an estuary at Anyanui and a lagoon at Narkwa. These sites were chosen because they were noted to be among the top oyster baskets of Ghana.

For oysters, about 30 pieces were handpicked from roots of mangroves and rocks from each location (n = 270) (Ada, Anyanui and Narkwa lagoon) and stored in an ice chest. Samples were subsequently transported to the laboratory, thawed, shucked and were depurated. The soft tissues were pooled, homogenized and transferred to bottles (glass, PTFE) and labeled according to the sampling sites. The homogenized samples were stored in a freezer below-20°C until chemical analysis.

For the sediment, each sampling site was demarcated into four and samples were collected using auger (n = 84) just around where the oysters were collected onto an aluminum foil and conveyed to the laboratory. Samples were then homogenized according to site, dried and sieved to remove other matrices.

Analytical reagents: Chromatography grade dichloromethane (HPLC grade, 99.8% purity, UN1593 EC: 200-838-9), n-hexane (purity (GC) $\geq$ 99.0%, analytical reagent, EC: 203-777-6, Product: 103876Q) and analytical grade acetone were purchased from VWR-BDH Chemicals Limited UK. Sodium sulphate (analytical Reagent, 99.4% purity, product: 28114.296) and glass wool were obtained from VWR-BDH PROLABO UK. Column chromatography silica gel (mesh: 70-230, product: 36020) was purchased from Auro Avenida Export, PVT Ltd (India). A PAH standard mixture containing 16 PAHs compounds (purity: 95.9-99.9%, 47940-U) was purchased from Supelco-analytical, Bellefonte, PA, USA. A mixture containing four isotopically labeled PAHs

used as internal standard (surrogate) were also purchased from Chem Service, West Chester, PA, USA.

Extraction of PAHS in oysters: Ten grams (wet weight) of blended oyster samples were extracted using cold maceration (with shaking) with 60 mL 1:1 hexane acetone mixture and 10 g of sodium sulfate anhydrous. Hexane acetone mixture extract containing organic pollutants was transferred to a 300 mL round-bottom flask with percolation through sodium sulfate anhydrous in a funnel. The extraction was repeated twice and all the hexane-acetone mixture extracts were combined. The extract was concentrated at 55°C to about 2 mL using Rotary evaporator (Rotavapor R-114)[11].

Clean-up procedure: The concentrated extract was cleaned off other matrices using packed silica gel column. The column used was prepared by loading 10 g of activated silica gel (130°C overnight) into a chromatographic column (all the columns used had uniform internal diameter of 1 mL). About 2 g of anhydrous sodium sulphate was added to the top of the column. Both ends of the packed column were plugged with glass wools. The column was precondition with 10 mL dichloromethane followed by 5 mL hexane. About 2 mL of the analyte was added onto the column and was first eluted with 15 mL hexane to remove saturated hydrocarbons which was subsequently discarded. This was followed by the addition of 20 mL (1:4) dichloromethane/hexane mixture to elute the PAHs. The final PAH extracts were concentrated to approximately 1 mL under a stream flow of nitrogen gas[11].

Extraction of PAHs in sediment: Ten grams of the sediment was homogenized with 10 g of anhydrous sodium sulfate and was extracted with 300 mL 1:1 hexane acetone mixture using Soxhlet apparatus. This was done for 16 h to ensure maximum extraction efficiency. Hexane acetone mixture extract containing organic pollutants was allowed to cool and transferred to a 300 mL round-bottom flask with percolation through sodium sulfate anhydrous in a funnel. This was repeated twice and all the hexane acetone mixture extracts were combined. The extracts were concentrated to about 2.0 mL and cleaned off other matrices using the clean-up procedure used for the oysters[11].

Instrumentation: Before the final instrumental analysis, each extract was spiked with 1.0 µL of working deuterated surrogate standards (2-Fluorobiphenyl and p-terphenyl-d14) deuterated PAHs consisting of naphthalene-d8, anthracene-d10, p-terphenyl-d14 and benz[a]anthracene-d12.

The identification of PAHs was conducted using Agilent 6890N GC-FID/MS operating in a selective split mode. The injection was done manually. A SLB5TM MS fused capillary column (30×0.25 mm i.d.×0.25 µm film thickness) and helium carrier gas at flow rate of 1.5 mL min^{-1} were used in the separation. The make-up flow of the helium carrier gas was 20 mL min^{-1} and an air flow of 300 mL min^{-1}. The temperature was programmed as follows: Oven set-point was 60°C, hold for 2 min, 40°C min^{-1} to 170°C, 10°C min to 220°C, 5°C min^{-1} to 290 hold for 10 min. The injections of 2 µL were performed in the split mode and the split valve was opened after 2 min. The split ratio was 50:1. Sample peaks were identified based on retention times on target ion chromatograms and in relative abundance of the qualifiers ions selected for each PAH in comparison with PAHs standards. Selective ion monitoring acquisitions were also done by comparing base peaks.

Analytical quality control: A modified extraction procedure[12] was employed in the recovery studies. Recovery study procedure was conducted to test the efficiency of the extraction system as well as GC-FID/MS. The recovery study also involved random spiking of the sediment and oyster samples with deuterated p-terphenyl surrogate standard solutions before extraction. Hundred micrograms per milliliter of PAH standard solution was added to the samples and extracted in the same way as the non-spiked samples. The extracts were analyzed and the recoveries were calculated from the differences in total amount of PAH standard spiked and the amount realized after analysis. Several deuterated PAH standards were used for the recovery calculation as directed by the method employed[13]. The analytical precision and recovery of the PAHs were also checked with NIST standard reference material (1941b) which is marine sediment collected at the mouth of the Baltimore Harbor intended for use in evaluating analytical methods for the determination of selected PAHs in marine sediments and similar matrices.

Calculating carcinogenic and mutagenic risk using benzo[a]pyrene toxicity equivalent factors (TEF) and mutagenic equivalent factors (MEF): Toxic equivalency factors (TEFs) have been developed for a number of individual PAHs classified as potential carcinogens by a number of

researchers and institutions, the factor for each of the PAHs expressing its potency relative to benzo[a]pyrene a well-known potent carcinogen, which has a TEF of unity[10]. The concentration of each of the individual PAH compounds is multiplied by its TEF (Table 1) and subsequently summed up to yield benzo[a]pyrene equivalent concentrations, TEQBaP[14].

By this means, the concentrations of a suite of PAHs can be represented by a single concentration, which reflects the overall carcinogenic potential of the PAHs within the sample for which TEFs have been assigned. This technique has in recent times been successfully applied in fresh seafood monitoring studies and other wider monitoring programmes[10,18]. The mutagenicity of individual PAHs relative to BaP has also been conducted using the mutagenic factor (MEF)[16,19] (Table 1). The sum of the concentration of each individual PAH multiplied the corresponding MEF gives the mutagenic equivalents (MEQ):

$$\text{TEQ-BaP} = \Sigma\,(\text{TEF}_i \times C_i) \quad (1)$$

$$\text{MEQ-BaP} = \Sigma\,(\text{MEF}_i \times C_i) \quad (2)$$

where, C_i is the measured individual PAHs concentrations for the 'ith' compound with the assigned TEF_i.

The TEF (for TEQ-BaP) and MEF (for MEQ-BaP) approach has been adopted in this study because PAH contamination rarely consists of a single compound but rather of mixtures of compounds that can affect the environment and human health[20,21]. The assessment of individual PAHs irrespective of their relative potency was believed to generate inaccurate or misjudged value for carcinogenic and mutagenic risk since it focuses on single compounds[10]. The calculated TEQ-BaP and MEQ-BaP for the seven U.S EPA classified carcinogens (mutagens) were used to estimate carcinogenic and mutagenic risk involved in ingestion of oysters used herein for life time of 70 years[22]. The total risk due to exposure to mixtures of carcinogenic (or mutagenic) PAHs is:

$$\text{Risk (carcinogenic or mutagenic)} = \left[\begin{matrix}\text{SF}_{\text{BaP}} \times \text{BaP equivalent} \\ \text{dose of mixtures of PAHs}\end{matrix}\right] \quad (3)$$

where, SF_{BaP} is the oral carcinogenic slope factor of benzo[a]pyrene (7.3 mg kg^{-1}/day). The BaP equivalent daily dose of compound 'i' is given as:

$$\text{BaP}_{\text{EQ}}\,\text{Dose}_i = \text{TEF}_i \times \text{Dose}_i \quad (4)$$

Hence the daily BaP equivalent dose of mixtures of carcinogenic (mutagenic) PAH compounds was calculated for carcinogenicity and mutagenicity using Eq. 5:

Table 1: Proposed benzo(a)pyrene equivalent factors for carcinogenicity (TEF) and Mutagenicity (MEF)

PAH	TEF[15]	MEF[16,17]
Chrysene	0.001	0.017
Benz[a]anthracene	0.100	0.082
Benzo[b]fluoranthene	0.100	0.250
Benzo[k]fluoranthene	0.010	0.110
Benzo[a]pyrene	1.000	1.000
Indeno[1,2,3-cd]pyrene	0.100	0.310
Dibenz[a,h]anthracene	1.000	0.290

$$\begin{matrix}\text{BaP equivalent dose of} \\ \text{carcinogenic (mutagenic) PAHs}\end{matrix} = \left[\frac{\text{TEQ (or MEQ)} \times \text{IR} \times \text{EF} \times \text{ED} \times \text{CF}}{\text{BW} \times \text{AT}}\right] \quad (5)$$

These exposure assumptions were made to be consistent with EPA guidance on default assumption on "reasonable maximum exposure[22]". Where IR is the ingestion or intake rate of carcinogenic (mutagenic) PAHs in µg/day, EF is the exposure frequency to carcinogenic (mutagenic) PAHs in days/year, ED is the exposure duration in years, CF is the conversion factor (i.e., 10^{-6} kg μg^{-1}), BW is the average body weight of Ghanaian adult in kg and AT is the average life time of 70 year expectancy. Mean ingestion rate of 89±20 g/day calculated based on a mean oyster's mussel of 46±15 g/meal consumed by the average Ghanaian adult was used. This was obtained through a structured interview conducted on 200 people randomly selected from the various communities engaged in this study. Exposure frequency of 350 days/year, exposure duration of 30 years and average adult body weight of 70 kg were used for the risk assessment. For risk associated with dermal contact to a mixture of PAHs in sediments, slope factor for benzo[a]pyrene of 25 mg kg^{-1}/day[23] and exposure frequency 52 days/year calculated based on about 3.5 h/day (approximately 1 day/week) was used. The following equation was used in conjunction with Eq. 3 to calculate the levels of incremental risks involved in dermal contact with mixtures of PAHs in sediments.

$$\begin{matrix}\text{BaP equivalent dose of} \\ \text{carcinogenic (mutagenic) PAHs}\end{matrix} = \left[\frac{\begin{matrix}\text{TEQ (or MEQ)} \times \\ \text{DA} \times \text{ESA} \times \text{EV} \times \\ \text{DR} \times \text{EF} \times \text{ED} \times \text{CF}\end{matrix}}{\text{BW} \times \text{AT}}\right] \quad (6)$$

where, dermal adsorption fraction (DA) = 0.13, Event frequency (EV) = 1 event/year and dermal adherence rate (DR) = 0.02 mg cm^{-2}/event[22]. The ESA is the exposed dermal surface area = 3067 cm^2 [22], EF, ED, CF, BW and AT have their usual meaning and values as stated earlier.

Statistical analysis: Microsoft excel's data analysis tool pack and SPSS 16.0 were employed for the data and statistical

analysis. Single factor ANOVA and two way ANOVA analysis conducted at 95% confidence level (CL) on triplicate results.

RESULTS AND DISCUSSION

Quality control results: The percentage recovery for the extraction and the GC-FID/MS analysis gave a good result which showed the efficiency of the method used. The percentage recovery for the individual PAHs and surrogates analyzed ranged from 50-119% and 76-128%, respectively (Table 2). The limit of detection and quantification used were 0.01 and 0.03 μg kg^{-1}, respectively. Single factor ANOVA analysis conducted at 95% confidence level (CL) on triplicate results for PAH showed statistically no significant difference. The result from the NIST-1941B reference material used for checking the efficiency of the extraction system and the GC-FID/MS instrument used had a recovery range of 62-101%. Analysis of variance of replicate results of each sample at the 95% confidence level showed no statistical significant difference.

Levels of PAH in oysters: The result (Table 3) shows the mean PAH concentration in the dry and wet seasons for the oysters from the various sampling sites (Ada, Anyanui and Narkwa). The mean total PAH concentrations in the oyster samples ranged between 66.85 and 168.59 μg kg^{-1} for the dry season whilst, that of the wet season ranged between 226.24 and 359.97 μg kg^{-1}, for the samples from Ada, Anyanui and Narkwa. There were elevated levels of PAHs in oyster samples from the sites studied during the wet season than the dry season and this may be attributed to the inflow of PAH polluted rain runoff waters from upstream which enter the water bodies during a pour. Thus, making available more PAH contaminated substances to be filtered or fed on by the oysters in the water body. This causes the PAHs to bio-accumulate in the oysters since they were known to filter larger volumes of water during this season.

The relatively low levels of PAH in oysters during the dry season as compared to the wet season may also be attributed to the fact, that, in the dry season, the sun's light (solar radiation) were normally high and most of the suspended PAHs (usually lower molecular weight PAHs) in the water

Table 2: Percentage recovery of some PAHs

Parameters	Recovery (%)
D10-Anthracene(sur)	128
D12-Benzo[a]pyrene(sur)	76
D8-Acenaphthylene(sur)	106
Terphenyl-D14(sur)	96
Acenaphthene	101
Acenaphthylene	114
Anthracene	96
Benz[a]anthracene	91
Benzo[b]fluoranthene	75
Benzo[k]fluoranthene	100
Benzo[ghi]perylene	82
Benzo[a]pyrene	83
Chrysene	119
Dibenz[a,h] anthracene	99
Fluoranthene	86
Fluorene	94
Indenol[1,2,3-cd]pyrene	71
Naphthalene	93
Phenanthrene	97
Perylene	104
Pyrene	85

Table 3: Mean PAH concentrations (μg kg^{-1}) in oysters (n = 3) during the dry and wet seasons for the various sampling sites

	Dry season			Wet season		
PAHs	Narkwa	Anyanui	Ada	Narkwa	Anyanui	Ada
Naphthalene	2.38	1.48	1.73	2.42	1.95	4.05
Acenaphthylene	2.22	6.61	2.86	7.99	7.30	10.80
Acenaphthene	3.78	10.52	5.77	2.20	6.36	0.70
Pyrene	8.32	20.24	9.95	60.95	40.59	137.00
Fluoranthene	6.33	45.82	6.99	151.80	98.80	99.60
Fluorine	7.27	10.40	6.25	12.88	11.65	10.70
Phenanthrene	4.73	4.12	2.68	9.80	6.96	7.09
Benz[a]anthracene	6.61	9.85	11.12	30.53	20.19	0.21
Chrysene	18.33	14.10	9.10	0.15	7.14	43.90
Anthracene	1.10	2.33	1.40	0.85	1.59	8.21
Benzo[b]fluoranthene	3.17	11.10	3.29	1.06	6.10	9.58
Benzo[k]fluoranthene	0.90	14.50	4.13	2.93	8.71	15.80
Benzo[a]pyrene	0.01	0.02	0.04	0.11	0.06	0.78
Indeno[1,2,3-cd]pyrene	1.69	14.20	2.38	0.170	7.19	6.89
Dibenz[a,h]anthracene	ND	0.02	ND	0.01	0.01	2.66
Benzo[ghi]perylene	0.01	3.22	0.33	0.05	1.64	1.07
Total	66.85	168.59	68.03	283.89	226.24	359.97

ND means value is below detection limit

Table 4: Mean concentration of PAH (μg kg^{-1}) in sediment (n = 3) during the dry and wet seasons for the various sampling sites

	Dry season			Wet season		
PAHs	Narkwa	Anyanui	Ada	Narkwa	Anyanui	Ada
Naphthalene	5.02	9.00	6.97	2.65	3.33	84.94
Acenaphthylene	7.88	6.25	5.99	4.94	3.98	0.79
Acenaphthene	4.90	4.76	6.58	8.79	5.53	0.83
Pyrene	ND	5.14	4.08	4.86	5.44	1.06
Fluoranthene	4.98	5.08	6.20	5.78	8.02	1.20
Fluorine	5.10	4.97	5.10	6.00	4.76	4.31
Phenanthrene	4.86	4.91	14.67	5.69	5.17	0.89
Benz[a]anthracene	7.05	9.04	8.78	4.86	4.69	1.53
Chrysene	5.01	4.07	4.69	5.73	5.80	0.84
Anthracene	7.94	6.54	5.99	7.11	8.07	9.53
Benzo[b]fluoranthene	4.99	7.00	17.85	4.35	4.69	6.47
Benzo[k]fluoranthene	5.07	8.09	4.55	4.14	5.35	0.62
Benzo[a]pyrene	4.98	4.99	7.24	0.05	4.57	1.24
Indeno[1,2,3-cd]pyrene	6.15	5.11	4.61	2.40	5.19	10.02
Dibenz[a,h]anthracene	ND	5.07	ND	4.67	5.63	0.11
Benzo[ghi]perylene	4.89	4.66	5.66	0.33	5.02	11.97
Total	78.82	94.65	108.97	72.35	85.24	136.35

ND means value is below detection limit

decomposed as they were photo sensitive and hence not available to aquatic organisms. Oros and Ross[24] reported a mean total PAH levels (16 PAHs) ranging between 184-689 μg kg^{-1} in oysters and Nakata *et al.*[25] also recorded a mean total PAH level of 230 μg kg^{-1} wet-weight in oysters from Tanoura Bay[22]. Earlier research conducted came to the conclusion that oysters truly accumulates a lot of PAHs pollutants[7]. The results obtained in this study were comparable to that obtained by Oros and Ross[24] and Nakata *et al.*[25]. However, how these oysters were used for determines its effect on the users. It was consumed heavily in Ghana, therefore, possible PAH bio-accumulation may cause serious health problems including cancers.

Benzo[a]pyrene, a well-known carcinogen usually used as biomarker for monitoring environmental PAHs, recorded values ranging from 0.01-0.78 μg kg^{-1} in oyster samples from Ada, Anyanui and Narkwa. According to the European Commission's Regulation (EC) # 208/2005, the maximum level of benzo[a]pyrene in bivalve molluscs should be 10.0 μg kg^{-1} wet weight[26]. Thus the levels of benzo[a]pyrene in oysters sampled in this study was very well within EC (2005) acceptable limit and may not pose any significant health risk to consumers. This also suggests that, water bodies from which oysters were obtained may not be significantly polluted with benzo[a]pyrene.

Analysis of variance (two-way ANOVA) conducted at 95% confidence level showed no statistical significant difference in PAHs levels in oysters between the three sites for a particular season ($p = 0.905$) and also between the two seasons for oysters from the three water bodies ($p = 0.112$). This suggests that PAH levels in oysters from the various water bodies in a particular season may not differ significantly from each other, hence similar pollution level may be observed across board. It also suggested that seasonal variation may not significantly affect the PAH levels in oysters from the various sites.

Levels of PAH in sediments: From the results (Table 4), the mean total PAH levels in sediments from the three towns (Narkwa, Anyanui and Ada) ranged from 78.82-108 μg kg^{-1}, in the dry season and 72.35-136.35 μg kg^{-1} in the wet season for samples from Ada, Anyanui and Narkwa. Contrary to the levels of PAHs in oysters from the same water bodies in these towns, generally, the dry season recorded elevated PAH levels in sediment samples than the wet season. This may be attributed to the fact that during the dry season the water bodies get dried up through evaporation and therefore, PAHs present in the water get concentrated, settle at the bottom and adhere to the surfaces of the water sediments. This may suggest an increased risk with respect to dermal contact to sediments from these waters during the dry season. Gilbert *et al.*[27], reported a total PAHs level range of 254-558 mg kg^{-1} in sediment samples from Fosu lagoon with a mean of 359.4 mg kg^{-1} [27]. Their results were far higher than the results obtained in this study, depicting the extent of pollution in the lagoon water when compared with the water bodies used in this study. This suggests that water bodies in this study were relatively less polluted.

A two-way ANOVA conducted at 95% confidence level showed no statistical significant difference between sites with respect to PAH levels in sediments from water bodies in these sites ($p = 0.078$). Implying, there is no appreciable difference in the extent of pollution in water bodies from the three

Table 5: Risk assessment based on carcinogenic and mutagenic equivalency (μg kg^{-1}), calculated for oyster ingestion during the wet and dry seasons in the three towns understudy

	Dry season			Wet season		
Carcinogenicity	Narkwa	Anyanui	Ada	Narkwa	Anyanui	Ada
Chrysene	0.0183	0.0141	0.0091	0.0002	0.0071	0.0439
Benz[a]anthracene	0.6610	0.9850	1.1120	3.0530	2.0190	0.0210
Benzo[b]fluoranthene	0.3170	1.1100	0.3290	0.1060	0.6100	0.9580
Benzo[k]fluoranthene	0.0090	0.1450	0.0413	0.0293	0.0871	0.1580
Benzo[a]pyrene	0.0100	0.0200	0.0430	0.1100	0.0600	0.7800
Indeno[1,2,3-cd]pyrene	0.1690	1.4200	0.2380	0.0170	0.7190	0.6890
Dibenz[a,h]anthracene	0.0050*	0.0150	0.0050*	0.0100	0.0140	2.6600
BaP-TEQ	1.1893	3.7091	1.7774	3.3255	3.5162	5.3099
BaPeq daily dose (μg kg^{-1})/day	0.6214	1.9380	0.9287	1.7376	1.8373	2.7744
Cancer Risk	4.5E-06	1.4E-05	6.8E-06	1.3E-05	1.3E-05	2.0E-05
Mutagenicity equivalency						
Chrysene	0.3116	0.2397	0.1547	0.0026	0.1214	0.7463
Benz[a]anthracene	0.5420	0.8077	0.9118	2.5035	1.6556	0.0172
Benzo[b]fluoranthene	0.7925	2.7750	0.8225	0.2650	1.5250	2.3950
Benzo[k]fluoranthene	0.0990	1.5950	0.4543	0.3223	0.9581	1.7380
Benzo[a]pyrene	0.0100	0.0200	0.0430	0.1100	0.0600	0.7800
Indeno[1,2,3-cd]pyrene	0.5239	4.4020	0.7378	0.0527	2.2289	2.1359
Dibenz[a,h]anthracene	0.0015*	0.0044	0.0015*	0.0029	0.0041	0.7714
BaP-MEQ	2.2805	9.8438	3.1256	3.2589	6.5530	8.5838
BaPeq daily dose (μg kg^{-1})/day	1.1916	5.1434	1.6331	1.7028	3.4240	4.4851
Mutagenic risk	8.7E-06	3.8E-05	1.2E-05	1.2E-05	2.5E-05	3.3E-05

Where: *Means PAH level was below detection limit (DL) and value was recalculated using ½ DL for use in risk assessment, BaP-TEQ and BaP-MEQ are the total benzo[a]pyrene toxicity equivalents for carcinogenicity and mutagenicity, respectively, BaPEQ = Benzo[a]pyrene toxicity equivalent

towns. For statistical difference between seasons of sampling with respect to PAH levels in sediments, significant difference ($p = 0.007$) was observed. Suggesting, the levels of PAHs in sediments of water bodies were seasonal dependent and the levels may be significantly high in the dry seasons.

Risk assessment

Risk involved in ingestion of oysters: While TEQ_{BaP} is directly associated with carcinogenicity, MEQ_{BaP} (mutagenic activity) may not be directly associated with cancer[10] and may have implications for other non-cancerous adverse health effects like pulmonary diseases, birth defects, impotency, infertility in humans, low IQ[10,28] etc.

From the result (Table 5), the total toxicity equivalencies for the seven U.S EPA priority carcinogens calculated ranged from 1.1893 (Narkwa) during the dry season to 5.3099 (Ada) during the wet season. The high TEQ-BaP obtained for oysters at Ada during the wet season poses potential carcinogenic risk when ingested. The corresponding BaPEQ daily doses and carcinogenic risk for an adult involved in a life time of 70 years ingestion of oysters from these sites were also calculated to be between 0.6214-2.7744 μg kg^{-1}/day for a risk of 4.5×10^{-6}-2.0×10^{-5}, respectively (Table 5). These risk values suggested that, for a life time consumption of oysters from Narkwa during the dry season, 45 out of 10,000,000 people are likely to suffer from cancer in their life time. Also, for the consumption of oysters from Ada during the wet season, 2 out of 100,000 people are likely to suffer from cancer once in their lifetime. The implication was that, the consumption of oysters from Narkwa in the dry season may not pose any significant carcinogenic risk to consumers since the risk value was within the U.S EPA unit risk of 1×10^{-5} whereas, consumption of oysters from Ada during the wet season may pose some carcinogenic risk, because its risk value was quite above the acceptable limit of 1×10^{-5}[29]. From the results (Table 5), it could generally be observed that elevated carcinogenic risk may be involved in the consumption of oyster during the wet season (risk $> 1\times10^{-5}$) than during the dry seasons (risk $< 1\times10^{-5}$). This may imply that oyster samples collected during the wet seasons may be unwholesome for consumption and may impart significantly on the health of consumers.

Also the mutagenic equivalences for these U.S EPA prioritized PAHs calculated ranged from 2.2805 for oysters from Narkwa (dry season) to 9.8438 for oysters from Anyanui (dry season). The corresponding BaPEQ daily doses were also calculated to be 1.1916 and 5.1434 μg kg^{-1}/day, respectively. Hence, the mutagenic risk involved in life time of 70 years ingestion of oysters from Narkwa and Anyanui during the dry season were calculated to range from 8.7×10^{-6}-3.8×10^{-5}, respectively. The implication again is that, for adult's life time ingestion of oysters from Narkwa and

Table 6: Risk assessment based on carcinogenic and mutagenic equivalency (μg kg^{-1}), calculated for dermal contact with sediments from the three towns during the wet and dry seasons

	Dry season			Wet season		
Carcinogenicity	Narkwa	Anyanui	Ada	Narkwa	Anyanui	Ada
Chrysene	0.0050	0.0041	0.0047	0.0057	0.0058	0.0008
Benz[a]anthracene	0.7053	0.9035	0.8780	0.4860	0.4690	0.1530
Benzo[b]fluoranthene	0.4992	0.6998	1.7850	0.4350	0.4690	0.6470
Benzo[k]fluoranthene	0.0507	0.0809	0.0455	0.0414	0.0535	0.0062
Benzo[a]pyrene	4.9760	4.9900	7.2400	0.0480	4.5700	1.2400
Indeno[1,2,3-cd]pyrene	0.6151	0.5110	0.4609	0.2400	0.5190	1.0020
Dibenz[a,h]anthracene	0.0050*	5.0700	0.0050*	4.6700	5.6300	0.1100
BaP-MEQ	6.8563	12.2593	10.4191	5.9261	11.7163	3.1590
BaPeq daily dose (μg kg^{-1})/day	0.0477	0.0853	0.0725	0.0412	0.0815	0.0220
Carcinogenic risk	1.1E-06	2.0E-06	1.7E-06	9.5E-07	1.9E-06	5.1E-07
Mutagenic equivalency						
Chrysene	0.0852	0.0691	0.0798	0.0974	0.0986	0.0143
Benz[a]anthracene	0.5783	0.7409	0.7200	0.3985	0.3846	0.1255
Benzo[b]fluoranthene	1.2480	1.7495	4.4625	1.0875	1.1725	1.6175
Benzo[k]fluoranthene	0.5577	0.8898	0.5005	0.4554	0.5885	0.0682
Benzo[a]pyrene	4.9760	4.9900	7.2400	0.0480	4.5700	1.2400
Indeno[1,2,3-cd]pyrene	1.9068	1.5841	1.4288	0.7440	1.6089	3.1062
Dibenz[a,h]anthracene	0.0015*	1.4703	0.0015*	1.3543	1.6327	0.0319
BaP-MEQ	9.3535	11.4937	14.4330	4.1851	10.0558	6.2035
BaPeq daily dose (μg kg^{-1})/day	0.0651	0.0799	0.1004	0.0291	0.0699	0.0431
Mutagenic risk	1.5E-06	1.8E-06	2.3E-06	6.7E-07	1.6E-06	9.9E-07

Where acronyms and * have same meaning as in Table 5

Anyanui, about 9 out of 1,000,000 and 38 out of 1,000,000 people, respectively are likely to suffer from non-cancer related diseases in their life time. Thus, ingestion of oysters from Anyanui during the dry season may pose some mutagenic health risk to consumer as value calculated[29] was above the unit risk of 1×10^{-5}. The oysters from Narkwa in the same season may pose little or no mutagenic risk to consumers as the calculated values were within the acceptable unit risk. Generally, all the oysters collected from the three sites for both dry and wet seasons except for Narkwa (dry season), recorded mutagenic risk values above the U.S EPA unit risk of 1×10^{-5}. This result may not be too good for heavy consumers of these oysters. It may pose significant mutagenic risk them.

Incremental risk associated with dermal contacts to PAHs in sediments: In Ghana, people who scavenge for oysters from sediments usually work without any protective apparel. Scavenging for oysters is one of the major economic activities for most of the people living in the various communities studied; making dermal exposure to PAHs from sediments to those engaged in these activities inevitable, hence, the need to calculate human health risk in this regard.

From the results (Table 6), the carcinogenic equivalencies for the seven U.S EPA prioritized PAHs calculated ranged from 3.1590-12.2593 in sediments from the samples. The corresponding BaPEQ daily doses through dermal contact with PAHs in sediments were also calculated to be 0.0220 and 0.0853 μg kg^{-1}/day. Hence, the carcinogenic risk involved in life time of 70 years dermal contact with sediments from Ada (during wet season) and Anyanui (during the dry season) were calculated to range from 5.1×10^{-7}-2.0×10^{-6}, respectively. These implies that about 5 out of 10,000,000 people are likely to suffer from cancer in their lifetime when dermally exposed to PAHs in sediments from Ada during the wet seasons while 2 out of 1,000,000 people are likely to suffer from cancer in their lifetime when dermally exposed to PAHs in sediments from Anyanui. From the results (Table 6), it could be said that dermal exposure to PAHs in sediments from all the study sites during both dry and wet seasons may pose little or no carcinogenic risk because risk values calculated[29] were below the unit risk of 1×10^{-5}.

Also, the calculated mutagenic equivalency for the seven U.S EPA prioritized PAHs ranged from 4.1851 in sediments from Narkwa (wet season) to 14.4330 in sediments from Ada (dry season). The corresponding BaPEQ daily doses through dermal contact with PAHs in sediments were also calculated to be 0.0291 and 0.1004 μg kg^{-1}/day, respectively. Hence, the Mutagenic risk involved in life time of 70 years dermal contact with sediments from Narkwa (during wet season) and Ada (during the dry season) were calculated to range from 6.7×10^{-7}-2.3×10^{-6}, respectively. These imply that about 7 out of 10,000,000 people are likely to suffer from non-cancer and

other cancer related diseases in their lifetime when dermally exposed to PAHs in sediments from Narkwa during the wet seasons while 2 out of 1,000,000 people are likely to suffer from non-cancer and other diseases in their lifetime when dermally exposed to PAHs in sediments from Ada. From the results (Table 6), it could be said that dermal exposure of humans to PAH in sediments from all the sites studied during both dry and wet seasons may pose little or no mutagenic risk, because risk values calculated were all below (or within) the acceptable US EPA unit risk of 1×10^{-5}.

Comparatively, the carcinogenic and mutagenic risks of PAHs in the sediments were slightly higher during the dry season than the wet season. Hence people who scavenge for oysters and other bivalves in such waters ought to take into consideration, the seasonal variation of PAH distribution in order to use protective apparels.

CONCLUSION

The appreciable levels of PAHs in oyster samples observed with its associated risk of which some were above the US EPA unit risk (1×10^{-5}) from the sites studied (wet season) may have a significant health impact on the exposed population (consumers) since PAHs are known to bio-accumulate in the body. The average ΣBaP-TEQ, its corresponding BaPEQ daily dose and the cancer risk calculated for the consumption of oysters from the sites studied especially in the wet season may be seen as one of the risk factors which may add on to the upsurge in cancer cases in Ghana as reported by the Cancer Control Division of Ghana Health Service and needs further studies.

SIGNIFICANCE STATEMENTS

This study showed appreciable levels of PAHs in bivalves, sediments and water which will go a long way to educate Ghanaians on the levels and some possible harmful effect of PAHs. In recent years, there has been increasing cases of cancers among Ghanaian women. Since this area of research lacks some attention in Ghana, this study will provide data to scientist, policy makers and the general public of Ghana on the levels of PAHs in some Ghanaian environments.

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