

Polycyclic aromatic hydrocarbons in fishes and some environmental samples on the coast of Ghana

EBENEZER OSEI-YEBOAH, REGINA APPIAH-OPONG*, MARK OFOSUHENE

Clinical Pathology Department of Noguchi Memorial Institute for Medical Research, South Legon Drive, Accra, Greater Accra, Ghana.

Tel.: +233-30-2940421, *email: antichama@yahoo.com

Manuscript received: 19 February 2020. Revision accepted: 24 May 2020.

Abstract. Osei-Yeboah E, Appiah-Opong R, Ofosuhen M. 2020. Polycyclic aromatic hydrocarbons in fishes and some environmental samples on the coast of Ghana. *Ocean Life* 4: 4-16. The shift in industrialization in our society, including mining of coal and minerals, drilling for oil has led to the accumulation of xenobiotic products and natural chemical substances. The activities from mining and drilling have continuously contributed to the release of polycyclic aromatic hydrocarbons (PAHs) and lead to their deposition in coastal environments. The process ultimately leads to bioaccumulation in plants and animals, creating dangerous pollutants. PAHs is a well-known cause of cancer, mutation, and embryonic pathologies. In Jubilee oil fields, Ghana, oil drilling is ongoing. However, onshore baseline environmental assessment of PAHs has not yet been performed. In this study, we performed an environmental assessment of some communities bordering the oil drilling fields to establish (i) levels of fish DNA adduct formation of fishes, (ii) levels of PHA in soil, plants, water, and fish. Several fishes from three study areas showed the presence of micronuclei as evidence in the examination of blood smears, although their mean micronucleated frequencies were below the threshold frequency of 15%. There was no statistical difference between their mean frequencies using one-way ANOVA analysis with a p-value < 0.05 except that of the data from one species, *Chloroscombrus chrysurus* (Linnaeus, 1766). Reverse-phase HPLC analysis of fish, water, plant and soil samples collected from six study sites was performed. Two fish species *Pomadasys incisus* (Bowdich, 1825) at Aboadze and *Thunnus alalunga* (Bonnaterre, 1788) from Dixcove recorded four and one PAH compound, respectively with concentrations above the maximum contaminant levels of 30 µg/kg set by the USEPA. The mean concentration of PAHs in water samples from two of the four study areas ranged from 1.4 to 1255 µg/L. All samples recorded concentrations above the threshold limit value of 50 ng/L set by the World Health Organization. Sixty plant samples were collected across the six study areas, and only *Erythrina senegalensis* DC. and *Ficus umbellata* Vahl recorded the presence of PAHs with concentrations in the range of 0.15-4.70 mg/kg. Soil samples were collected from two different depths of 0-15 cm and 15-30 cm. The mean concentration of PAHs in surface soils (0-15cm) ranged from 0.1 to 95 mg/Kg, with that at 15-30 cm ranging from 0.12 to 105 mg/kg. The PAH composition profile in all the samples was similar, with 2- 3 ring PAHs being dominant, which is suggestive of a petrogenic source.

Keywords: biomarker, DNA adduct, fish, polycyclic aromatic hydrocarbon

INTRODUCTION

Crude oil is commonly regarded as one of the highest valued resources a country can possess because it plays a very important role in modern society. The high value of oil is because this commodity is currently the dominant energy source and is expected to remain so over the next several decades (Wang et al. 2006). Moreover, the oil demand kept increasing, thanks to the burgeoning populations worldwide, more industrialized countries, and manufacturing places with high demand for energy and crude oil (Madlener and Sunak 2011).

In huge quantities, Ghana has discovered crude oil off the shores of its Western Atlantic Coast. This discovery came after a century of exploration activities with financial support from various resources (Ecobank 2014). However, Ghana must wait three decades to claim the oil discovery in 2007 by the Jubilee partners because the discovery coincided with the Jubilee anniversary after Ghana declared independence from the UK in 1957. Thus, the first well was christened Jubilee and the Jubilee partners include Sabre Oil, Tullow Ghana Limited, and Gas Limited, and

Kosmos Energy Ghana. In October 2009, the reserves of the Jubilee fields were estimated at 490 million barrels of high-quality oil, and currently, an average of 102,630 barrels of oil are drilled daily (World Bank 2009). Total oil revenue from January to September 2013 amounted to GH¢1,150.2 million, against a target of crude oil production from the Jubilee field, averaging 102,503 barrels of oil per day (bopd).

The toxic composition of crude oil cannot be ignored because crude oil is a complex mixture of hydrocarbons containing more than 17,000 compounds (Pampanin and Sydnes 2013). Crude oil is composed of a group of substances called polycyclic aromatic hydrocarbons (PAHs) from two to eight conjugated ring systems and exhibits all the chemical properties of aromatic compounds. PAHs can have a range of substituents such as nitro, alkyl, and amino groups in their structure (Pampanin and Sydnes 2013). The precursors for PAHs found in crude oil are natural products such as steroids that have been chemically converted to aromatic hydrocarbons over time (Feng et al. 2009). PAHs can be formed naturally by low-temperature, high-pressure reactions of natural organic matter and, in

this way, make up a significant fraction of petroleum hydrocarbons (Latimer and Zheng 2003; Mishra and Das 2017).

In substantial concentrations, PAHs in the marine environment are divided into two groups based on their origin, namely pyrogenic and petrogenic (Pampanin and Sydnese 2013). The petrogenic PAH is derived from oil and drilling activities such as oil spills, disasters, and effluence from industrial sites, oil refineries, and the majority from traffic exhaust emissions. Meanwhile, the pyrogenic PAH is derived from volcanic eruptions, forest fires, and incineration. Pyrogenic PAHs are usually composed of larger ring systems (above 6 rings), while petrogenic PAHs are composed of 2 to 6 rings. The PAHs pollutants from the coastal areas and their water bodies primarily originated from effluent released by industries, runoff from roads, smelter industries, and oil spills, as well as water that is generated from drilling routine activities.

Oil seeps refer to natural springs where liquid and gaseous hydrocarbons trickle out of the ground, scattering all over the globe with a higher concentration in some regions of the world. The presence of a natural oil seep often has resulted in the discovery of oil reservoirs that are large enough to supply commercial oil production (Devold 2013).

During oil drilling, various chemicals and materials are released into the environment, e.g., drilling mud, drill cuttings, oil, and chemicals injected into the drilling machine to control corrosion or assist the separation of oil from water as well as general industrial waste (Adewole et al. 2010). Spillage or discharge of these wastes into the sea and/or land has been associated with adverse environmental and public health effects (Lyons et al. 1999). Their adverse environmental effects include the dead and moribund marine animals, the oil coating of shorelines and other water bodies and PAH contamination (particularly coastal waters, plants, and other biological organisms). The percentage of PAH in crude oil ranges between 0.2% and 7%. Some PAHs are bioaccumulative, persistent and toxic to humans and other organisms. In addition, the substances are carcinogenic, mutagenic, or teratogenic. Substances that combine these characteristics raised a serious concern for public health and the environment (German Federal Environment Agency 2012).

Therefore, tracking the levels of PAH in coastal zones is necessary. In this regard, biomarkers become crucial as they serve as indicators of PAH pollution. Fishes are suitable model organisms for environmental genotoxic bio-indicator organisms due to their role in the aquatic trophic chain. In addition, fish are sensitive to low concentrations of genotoxic substances (Al-Sabti and Metcalfe 1995). Several assays among them can evaluate the genotoxic effects of PAH on fish is micronuclei analysis in peripheral blood erythrocytes (Al-Sabti and Metcalfe 1995). The micronucleus assay is a quick, sensitive and reliable assay to determine damage to the DNA. Micronuclei can originate both from whole chromosomes delayed during cellular division anaphase and from acentric fragments resulting from chromosomal breaks which are not incorporated into the main nucleus (De Lemos et al. 2008). Genotoxicity results from DNA damage and can happen

through the formation of DNA adducts. DNA adduct formation is the covalent binding of highly electrophilic chemicals to DNA. Thus, altering DNA structure and making it unable to undergo normal processes of replication, transcription, and repair. If the proper DNA synthesis is not restored by repair mechanisms and the adducts persist, these alterations may cause mutations and cancer development.

Specific objectives of this study were: (i) To assess hepatic DNA adduct formation in selected fish species. (ii) To determine the PAH levels in fish harvested in the communities. (iii) To assess the levels of PAH in water, plants, and soil samples from the selected communities.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals and reagents used in this study include PAH Mix 14 Standard (from Dr. Ehrenstorfer GMBH Augsburg, Germany), Silica Gel (Merck, Germany), Acetone (Prolabo VWR BDH, Singapore), Dichloromethane (DCM), Hexane, Ethanol, Methanol, Maygrunwald, DPX Mountant, Acetonitrile, and Giemsa Stain (Sigma Aldrich St Louis, USA), Copper (II) Sulfate Pentahydrate [$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$], Sodium Sulfate Anhydrous [Na_2SO_4], Phosphoric Acid [H_3PO_4], were obtained from Wako Pure Chemicals, Japan.

Study area

The study was conducted in six districts bordering the marine environment of the Jubilee Oil field, Western Region of Ghana (Figure 1). The Western Region forms about 10% of Ghana's total land area; it covers an area of 23,921 km² (Western - Government of Ghana 2015). It is located in the south-western part of Ghana and shares borders with the Republic of Cote D'Ivoire to the west, Central Region to the east, Ashanti and Brong Ahafo Region to the north, and the Gulf of Guinea to the south. The Cape Three Points, which is the southernmost part of Ghana where crude oil was discovered in commercial quantities in June 2007, is located in this region. The western coastline covers approximately 95 km of stable shoreline, which extends from the Republic of Cote D'Ivoire borders to the estuary of Ankobra.

The 2010 population and housing census estimated the region's total population to be 2,376,031, representing 9.6% of the total population of Ghana (Ghana Statistical Service Report 2012). The region has about 75% of its vegetation within the high forest zone of Ghana, and its south-western parts are noted for the rainforest, interspersed with patches of mangrove forest along the coast and coastal wetlands (Western-Government of Ghana 2015). The region lies in the equatorial climatic zone characterized by moderate temperatures from 22°C to 34°C at night and day, respectively. The region is the wettest part of Ghana, with a double maxima rainfall pattern averaging 1,600 mm per year. The two rainfall peaks fall between May to July and September to October. It also experiences intermittent minor rains all year round. The humidity of the region ranges from 70-90%.

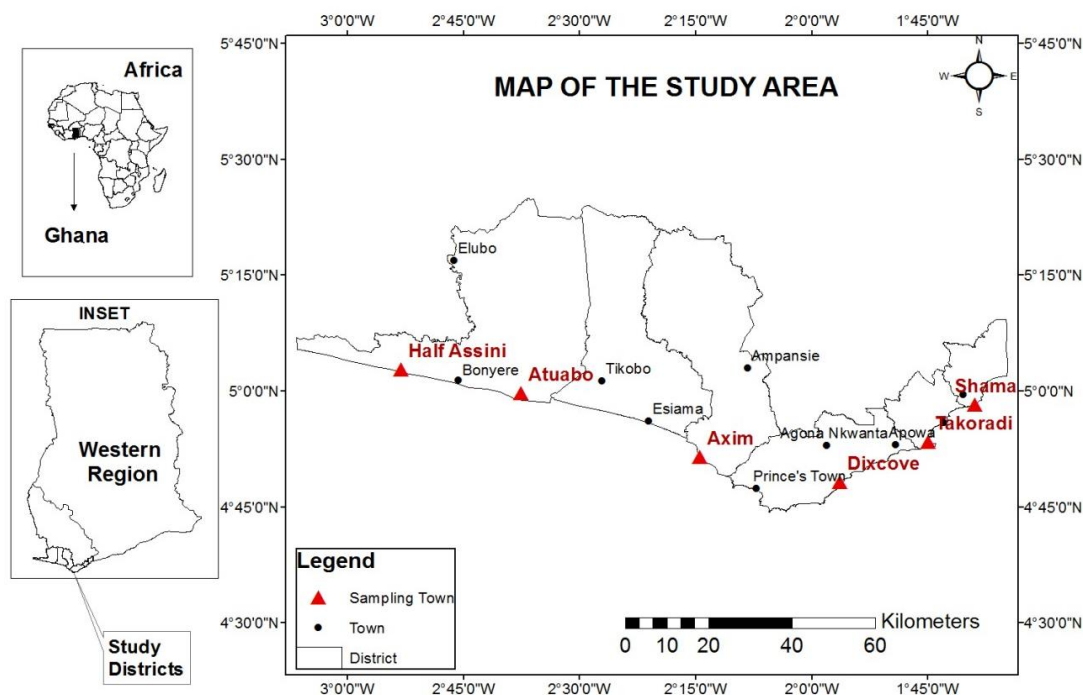


Figure 1. A map of the study areas in the Western coast of Ghana

The major industrial activities in the region are petroleum industry, oil and gas exploration, services, agriculture, excluding fishing but including forestry and hunting, mining and quarrying, manufacturing, and wholesale and retail trade (Western-Government of Ghana 2015). The region is also endowed with a wide variety of minerals, including gold, bauxite, iron, diamond, and manganese, and thus the region is an attractive investment destination for numerous small and large-scale gold mining companies.

Ellembelle District

The district covers a total land area of 1468 km² and is located in the southern end of the region between longitudes 2°05' W and 2°35' W and latitudes 4°40'N and 5°20'N. It shares boundaries with Jomoro District to the west, Nzema East Municipal to Tarkwa - Nsuaem Municipal to the East, Wassa Amenfi West District to the north, and a 70 km stretch of sandy beach to the south. The district lies within the wet semi-equatorial climate zone. According to the 2010 Population and Housing Census, the district's population is 87,501 (Ghana Statistical Service Report 2012). The area experiences rainfall throughout the year, with the highest monthly mean rainfall occurring between May and June. The mean annual rainfall is 1,600 mm, and the average temperature is about 29°C. Fishing is the major industry in this district. The area's vegetation comprises moist semi-deciduous rainforest and secondary forest southward with savannah vegetation along the coastal area. The topography is generally undulating, with the highest point at about 450ft above mid-sea level.

Jomoro District

The district, covering a land area of 1344 km², is located between latitudes 04° 55' N and 05° 15' N and longitudes 02° 15' W and 02° 45' W. It shares boundaries with Aowin-Seaman and Wassa Amenfi to the north, Ellembelle District to the east, La Cote D'Ivoire to the west and the Gulf of Guinea to the south. The population of Jomoro District, according to the 2010 population census, is 150,107 (Ghana Statistical Service Report 2012). The heavy rainfall occurs from April to July and September to November. The average annual rainfall is 1732 mm. There is also a short dry spell in August and a long dry period from December to January. The climate of the area is classified as equatorial monsoon. The vegetation is a tropical rainforest with coastal vegetation, largely mangrove swamps (Western-Government of Ghana 2015). Jomoro District is basically an agricultural one, and arable farming and livestock rearing constitute the backbone of her economy. Agriculture engages the majority of the population who obtain their livelihood from farming and other agro-related activities such as deep-sea and freshwater fishing as well as coconut oil extraction.

Nzema East Municipality

Nzema is located at the southern end of the region between longitudes 2° 05' and 2° 35' W and latitudes 4° 40' and 5° 20' N of the equator. It is bordered on the west by Ellembelle District, on the north by Wassa Amenfi West District, on the east by the Tarkwa Nsuaem Municipal, Prestea Huni Valley, and Ahanta West Districts, and on the south by the Gulf of Guinea. It covers a land area of 2194 km². The population of the district is 60,828, according to

the 2010 Population and Housing Census (Ghana Statistical Service report 2012). It lies between the wet semi-equatorial climatic zones. Rainfall is experienced throughout the year, with the highest monthly mean occurring around May and June each year. The average temperature is about 29°C. The surrounding vegetation is made up of moist semi-deciduous rainforest, mainly in the northern part, with secondary forest southwards. The topography is generally undulating, with the highest point of about 100 m above sea level. This district is predominantly a fishing community. The main landing beaches are Lower Axim, Ahobre, and Effasu.

Ahanta West District

The district lies between latitude 4°45 'N and longitude 1°58 'W. It covers a total land area of 591 km², and 106,215 people occupy it, according to the 2010 Population and Housing Census report (Ghana Statistical Service Report 2012). It is bounded on the east by the Sekondi Takoradi Metropolitan Assembly (STMA), on the west by the Nzema East Municipal, and on the north by Mpohor Wassa East and Tarkwa Nsuem Districts and the Gulf of Guinea to the south. The area's climate is within the south-western equatorial climate zone, with the highest mean temperature of 34°C recorded between March and April and the lowest mean temperature of 20°C in August. It experiences a double maxima rainfall of over 1700 mm with an average relative humidity of about 75%. The vegetation falls largely within the high rainforest vegetation zone. The topography is generally low-lying.

Shama District

It covers a land area of 215 km² and is bordered to the north by the Mpohor Wassa East District, to the south by the Gulf of Guinea, to the west by Sekondi-Takoradi Metropolitan District and the east by Komenda Edina Eguafo-Abirem District in the Central Region. The district has a population of 81,906 (Ghana Statistical Service Report 2012). It lies within the tropical climatic zone. The area experiences two rainfall maxima each year. The major rainy season starts from March to mid-July, followed by a short dry spell that runs till August. The period from early September to mid-November marks the minor rainy season. The annual rainfall varies from 1000 to 1700 mm, with the mean annual rainfall of about 1380 mm. The annual temperature ranges from 25 to 28°C. The vegetation is mainly coastal thicket, thin to dense shrubs. The northern part of the district is made up of thick bushes with other small-sized trees. In the coastal area, the thicket is intermingled with tall grass species, including mangrove swamps and raffia groves at the estuary of the river Pra. It lies within a low-lying area, and the landscape is generally undulating with an elevation in most parts less than 80 m above sea level. The geological formations are made up of Lower Birimian and granite soils. The coastal areas are parent materials with faulty shelves and sand of various types resting on granite, gneiss, and schist (Western-Government of Ghana 2015). The Volta River Authority's thermal generation plant, which generates electricity from diesel and gas, is located in this district.

Sekondi-Takoradi Metropolitan District

The district, which covers a land area of about 49.78 km², is located in the southwestern part of Ghana. It is bordered to the north by Mpohor Wassa East District, east by Shama District, west by Ahanta West District, and south by the Gulf of Guinea. The metropolis's population is 559,548, according to the 2010 Population and Housing Census (Ghana Statistical Service Report 2012). The climate is equatorial, with an average annual temperature of about 25°C. Rainfall is bi-modal, with the major season occurring between March and July and the minor season occurring between August and November. The mean annual rainfall is about 1,380 mm. The natural vegetation is mainly woodland with thickets interspersed with tall grass species along the coastal areas (Western-Government of Ghana 2015). The area has a varied landscape and is undulating with ridges and hills. The area's geology is underlain with faulty shale and sandstone on granite, gneiss, and schist (Western-Government of Ghana 2015).

Experimental design

Communities were clustered into three according to distance from the Jubilee field: over ten kilometers (Aboadze in Shama District and New Takoradi in Sekondi-Takoradi Metropolis) onshore of the oil fields, 5-10 kilometers (Atuabo in Ellembelle District and Half Assini in Jomoro District) and under-five kilometers (Dixcove in Ahanta West District and Lower Axim in Nzema east Municipality). Samples were collected from a randomly selected community in each district. Five sampling sites were randomly selected in the community for plant and soil collection. Water samples were collected from five different sources in each community (where surface water or wells were found), and fish samples were obtained from landing beaches in each community.

Inclusion and exclusion criteria

This study site covered the coastal communities bordering the Jubilee oil field in the Western region of Ghana. We exclude water samples from water bodies because they did not serve any domestic purpose in each particular study area. Instead, leaves were collected from dominant plant species in each community. Consequently, only fresh fish were obtained from the various landing beaches in each study area.

Sample collection **Soil samples**

Soil samples were obtained from 5 different locations from the in-land and shores of selected susceptible communities at depths of 0 to 15 cm and 15 to 30 cm (each study area represented by 10 soil samples). The samples were stored in plastic containers for PAH extraction and analysis.

Water samples

Water was collected from 5 different locations except for Shama and Sekondi-Takoradi Metropolitan Districts, transferred into clean bottles and stored at 4°C until PAH analysis was performed. Shama and Sekondi-Takoradi Metropolitan Districts were excluded because they did not

meet the criteria as water from these sources was not used for domestic purposes. A total of 20 water samples were collected from five sources in each of the four study areas.

Plant and fish samples

The dominant plant samples were randomly taken from areas in selected communities near the in-land and shores. They were kept in sealed plastic bags and stored at 4°C until utilized for PAH analysis. For each sampling cluster, two different plant species were collected, thus, 10 plant samples were collected from each study area, and a total number of 60 plants were taken for this project.

The number of fish species sampled differed from district to district, but in all 38 fish samples (three per each species making a total of 114) were collected from the entire selected cluster or fishing community for PAH extraction and micronucleus test. The samples were transported on ice to the laboratory and stored at -20°C until use. A total of 27 fish species were collected for this project, but some species were found in more than one study area, thus the total number of 38 fish samples.

Determination of PAHs concentration

Polycyclic aromatic hydrocarbons (PAHs) concentration in plants, soil, and water samples were analyzed according to the method described elsewhere (Li et al. 2008). Meanwhile, the PAH content in fish was modified from the method described by Denton et al. (1999).

Preparation of samples

Plants

The plant sample was ground into a homogenous mixture using a pestle and mortar, then two grams of the samples were placed in a conical glass flask containing 5 mL acetone, 5 g of anhydrous sodium sulfate, and 0.1 g copper powder. Extraction was done in an ultrasonic water bath at a temperature of 30°C for 15 min. The mixtures were centrifuged at 2190 rpm for 10 min. Next, the supernatant was collected into fresh 15 mL centrifuge tubes. The extraction process was repeated by adding 5 ml of acetone to the residues, and the content was transferred into glass flasks before sonicating. The supernatants were then pooled together and evaporated in a rotary evaporator, then subjected to further extraction by column chromatography.

Soil

Soil samples were air-dried at room temperature (25°C), gently crushed, and filtered using a 1 mm mesh sieve to remove the larger size of particles. One gram of each sieved soil sample was transferred in a 100 mL glass beaker containing 5 mL acetone, 5 g of anhydrous sodium sulfate, and 0.1 g copper powder. The extraction process was the same as done in section 2.2.5.1.1.

Fish

Fish livers (1.5 g) were thawed prior to extraction. Subsequently, 10 g anhydrous, 10 ml of dichloromethane, and granular sodium sulfate were added to each tissue

sample before homogenization using a mortar and pestle. The mixture was centrifuged at 3500 rpm for 20 min. Next, the supernatant was collected into a new 50 mL centrifuge tube. The process was repeated and the supernatants for each sample were pooled. After reducing the volume to approximately 0.5 ml using rotary evaporation, the extract was transferred to a 15 mL centrifuge tube with two 0.5 mL rinses of dichloromethane. Next, the tube was placed in a water bath and the extract volume was reduced to 0.25 mL under a gentle stream of nitrogen. Each extract was then dissolved in 2 mL of hexane, and a further reduction in volume to 1 mL was done before further extraction by column chromatography.

Water

Before extraction, the C18 cartridges were pre-conditioned with 5 mL acetonitrile, followed by vacuum drying for 10 sec and washed with 5 mL of HPLC grade ultra-pure water. PAHs extraction using a solid-phase extraction (SPE) cartridge system. Each water sample (100 mL) was then percolated through the cartridges at a flow rate of 5 mL/min with a vacuum pump. The column was then eluted isocratically with 10 mL hexane and dichloromethane (1:1 v/v). The eluent of each sample was then subjected to HPLC analysis.

Column chromatographic extraction of PAHs

All the extracts from plants, soil, and fish samples were subjected to further extraction of PAHs by column chromatography.

Plant, soil and water samples

Ten glass columns (Ø1.5 mm) were packed with cotton wool by positioning the cotton securely in the narrowest part of the column using a long glass rod. The columns were securely clamped and supported on a stand, after which the taps were closed. This step was followed by filling the columns to one-third of their volume with dichloromethane and hexane (1:1 v/v) as a mobile phase. First, the silica slurry was prepared by mixing 70 g of silica gel and 200 mL of mobile phase in a glass beaker. The slurry was then pipetted into the column with a Pasteur pipette, and the solvent was allowed to drain to prevent overflowing. Next, the columns were gently tapped with a rubber bung to free them of air bubbles. This step was followed by draining the solvent until its level was even with the surface of the stationary phase, after which the tap was closed. The concentrated samples were then dissolved in 2 mL of the mobile phase. Next, 1 mL of each sample was loaded into a separate packed column. Next, each sample was eluted with 8 mL of mobile phase, and the eluents were collected in labeled test tubes. This was followed by evaporating the eluted samples to dryness under a gentle flow of nitrogen gas. Finally, the residue was reconstituted with 0.5 mL of acetonitrile for HPLC analysis. Further extraction of PAHs in soil and fish samples by column chromatography was done as described above.

HPLC analysis of samples

All samples were analyzed by the HPLC (Shimadzu from Japan) using a C18 reverse-phase column coupled with an ultraviolet detector. The separation of analytes was achieved by adopting the following conditions, a flow rate of 1.0 mL/min at 40°C. The injection volume was 20 µL. The column was stabilized at 40°C for 1 hour before chromatography. The mobile phase comprised water, phosphoric acid (component A), and acetonitrile (component B). Details of the mobile phase are given in Table 1. Analyte (PAHs) peaks were identified by their retention times compared to the corresponding retention times of the PAH standards used.

Genotoxicity assessment

Genotoxicity assessment of fish samples was done using the Micronucleus assay of Garg et al. (2012).

Micronucleus assay

Blood smears samples were made using peripheral blood samples from the caudal vein of the fish specimen. The slides were then air-dried at room temperature for 24 h. Dipping slides fixed slides into methanol for 5-10 min. The slides were then smears were stained with Maygrunwalds stain solution-I for 2-3 min, washed with double distilled water and dried. Upon drying, smears were stained with Maygrunwalds stain solution-II for 3-6 min, washed with double distilled water and dried. Finally, all the slides were then stained with Giemsa stain (10%) in phosphate buffer for 30 min and washed with double distilled water to remove all Giemsa particles. The prepared slides were fixed with DPX mountant and dried overnight. Normal and abnormal cells and micronucleus were observed and counted under a microscope (Leica, Germany) at a magnification of 100×.

The average micronuclei per 1000 cells of each fish species were determined based on the following scoring criteria; (i) The micronucleus resembles the staining characteristics and possesses similar morphology to the main nuclei. (ii) Any micronuclei present in the cytoplasm or only just touching the main nucleus. (iii) Micronuclei were smooth-edged and smaller than one-third the diameter of the main nuclei. Then, the frequencies of micronuclei were calculated for each species using the equation below.

$$\text{MNE } (0/100) = \frac{\text{Number of cells containing micronuclei}}{\text{Total number of cells counted}} \times 1000$$

Statistical analysis

Results were expressed as means + standard deviation (SD). Concentrations of plants and soils were expressed as mg/kg wet weight, whereas concentrations of fish were expressed as µg/g. Water concentration was shown as µg/L. The results of the micronuclei test were expressed as mean micronuclei frequency/1000 cells. Comparisons of micronuclei frequency/1000 cells between species across study areas were made using one-way analysis of variance (ANOVA) with the statistical package SPSS 14.0.2 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Concentration of PAHs

Concentration of eighteen PAH compounds namely, naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluorene, fluoranthene, pyrene, anthracene, benz[a]-anthracene, benz[a]fluoranthene, benz[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, chrysene, and indeno[1,2,3,c,d] pyrene were determined in water, fish liver, plants and soil samples.

PAHs in fish livers

The number of analyzed fish species from each district differed from the various landing beaches in the selected communities. The means and standard deviation of PAHs in all the fish species were tabulated from the data obtained from duplicate HPLC analysis of composite samples of each fish species (each species represented with three fish). For HPLC analyses, the liver of three fishes for each species was pooled together for extraction.

Nine different fish species (three per each species) namely: *Illisha africana*, *Sardinella aurita*, *Sardinella eba*, *Brachydeuterus auritus*, *Dentex congensis*, *Pomadasys incisus*, *Pseudupe prayensis*, *Lutjanus fulgens* and *Katsuwonus pelamis* were sampled from Shama District in the Aboadze landing beach. Only *P. incisus* detected acenaphthylene (15.267 µg/g), naphthalene (6.933 µg/g), 2-methylnaphthalene (5.667 µg/g) and acenaphthene (0.333 µg/g) (Figure 2). No PAHs were detected in the other fish species from the community. From Dixcove in the Ahanta West District, the fish species *Thunnus albacores*, *Trichiurus lepturus*, *Thunnus alalunga*, and *K. pelamis* were sampled. However, only fluorene with a concentration of 0.180 µg/g was detected in *T. alalunga* fish (Figure 2).

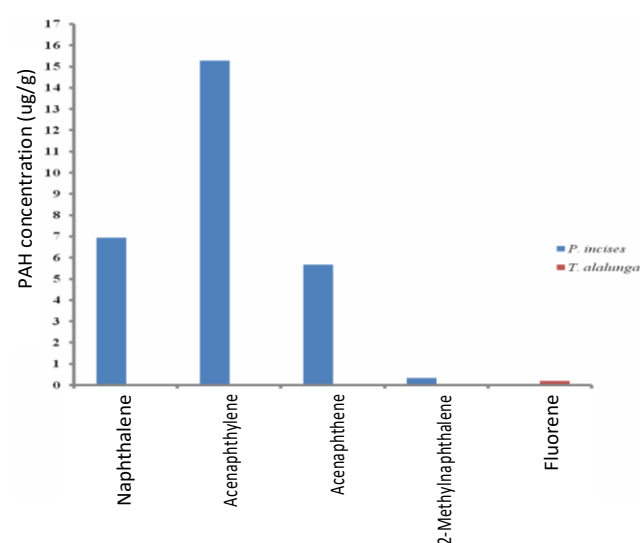


Figure 2. PAH concentrations in *P. Incises* liver from Aboadze in Shama District and *T. alalunga* fish from Dixcove in Ahanta West District, Ghana

Table 1. The mobile phase composition

Time/(min)	0.1% Phosphoric acid in water (%)	Acetonitrile (%)
0	50	50
5	60	40
10	80	20
25	80	20
30	95	5

Fish species: *Pentanemus quinquarius*, *Galeoides decadactylus*, *Pseudolithus senegalensis*, *I. africana*, and *B. auritus* from New-Takoradi landing beach in the Sekondi-Takoradi Metropolitan District showed no detectable PAHs. No PAHs were detected in the fish species: *B. auritus*, *Dentex angolensis*, *Chloroscombrus chrysurus*, *Sphyræna sphyræna*, *Stromateus fiatola* and *Pseudolithus typus* sampled in Lower Axim in Nzema East District.

Furthermore, no PAHs were found in *Seriola dumurili*, *Caranx chrysurus*, *Ethmalosa dorsalis*, *S. sphyræna*, *Caranx hippos*, and *Selene dorsalis* fish species sampled from Atuabo in the Ellembele District. Likewise, no PAHs were detected in fishes: *B. auritus*, *S. Sphyræna*, *Hemiramphus brasiliensis*, *S. aurita*, *Pomadasys incisus* (Bowdich, 1825) and *Acanthurus monroviae* from Half Assini in the Jomoro District.

Livers from each three *P. incisus* and *T. alalunga* were pooled and extracted with dichloromethane (DCM), then subjected to silica gel column chromatography prior to duplicate reverse phase HPLC analysis. Concentrations were calculated relative to PAH standards. Results are means of n= 2.

PAHs in water samples

Inland water bodies, including rivers and dug wells used for domestic purposes, were analyzed for the water PAHs. Samples were collected from four out of the six study sites/ communities. These four communities are Atuabo in Ellembele, Lower Axim in the Nzema East, Dixcove in Ahanta West District and Half Assini in the Jomoro District. However, water samples from Atuabo and Half Assini had no PAH detected in them.

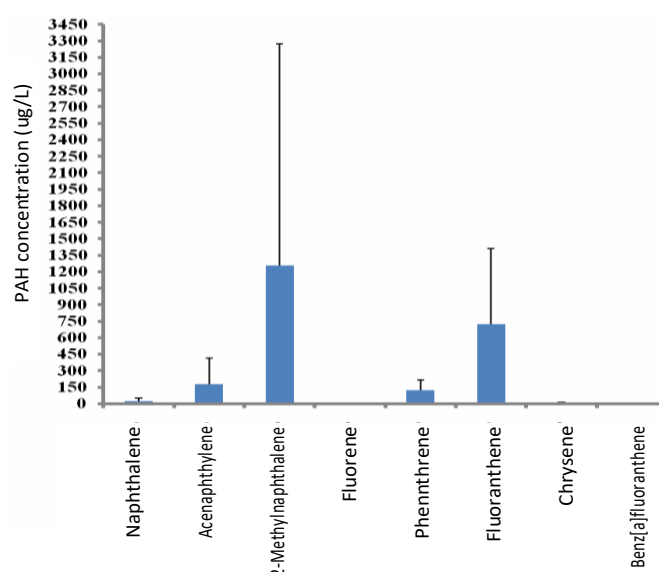
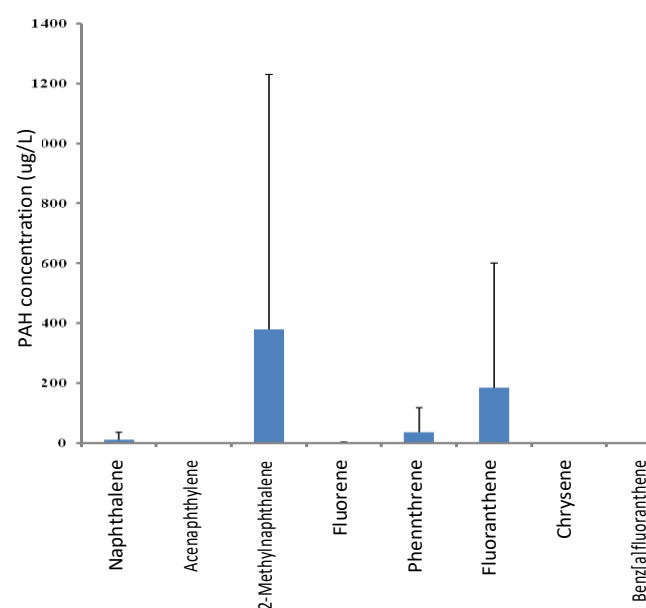
In Dixcove, 1-methyl naphthalene, naphthalene, acenaphthylene, fluorene, phenanthrene, benz[a] fluoranthene, fluoranthene, and chrysene were detected with an average concentration of 21.20 ± 29.85 , 178.50 ± 253.95 , 1255.30 ± 2015.40 , 2.40 ± 2.55 , 124.30 ± 92.55 , 723.40 ± 685.90 , 3.10 ± 6.95 and 2.20 ± 4.90 $\mu\text{g/L}$, respectively (Figure 3). Water bodies from Half Assini had the following PAHs: 1-methyl naphthalene, naphthalene, fluorene, fluoranthene, phenanthrene, detected with an average concentration of 11.4 ± 25.45 , 380.3 ± 850.0 , 1.4 ± 3.1 , 36.7 ± 82.05 , 185.5 ± 415.00 $\mu\text{g/L}$, respectively (Figure 4).

Water samples from five water bodies in this study site were subjected to solid-phase extraction and prior to reverse phase HPLC analysis after which concentrations of each PAH compound was calculated relative to

concentrations of PAH standards. Results are means $\pm$ SD. N=5 (Figure 3-4).

PAHs in plants samples

A total of sixty plants (ten from each district) were collected for PAHs analysis with the common ones being *Alchornea cordifolia*, *Chromolaena odorata*, *Avicennia nitida*, *Erythrina senegalensis*, *Ficus sagittifolia*, *Ficus exasperata*, *Ficus umbellata*, *Terminalia catappa*, *Spondias mombin*, *Thespesia populnea*, and *Senna siamea* various sampling clusters in the study sites. The means and standard deviation of PAHs in plant leaves were computed from the data obtained from duplicate HPLC analysis of composite samples of leaves of each plant species.

**Figure 3.** PAH concentration in water samples from Dixcove in Ahanta West District, Ghana**Figure 4.** PAHs concentration in water samples from Half Assini in Jomoro District, Ghana

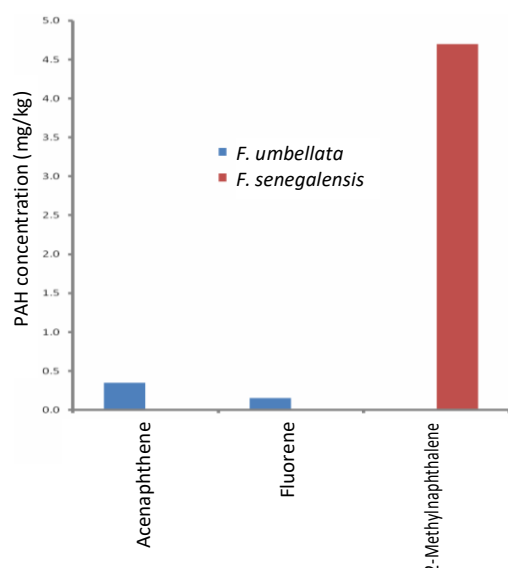


Figure 5. PAHs concentration in plant samples from Lower Axim in Nzema East District, Ghana

From all samples, *E. senegalensis* and *F. umbellata* from Lower Axim in Nzema East District had some PAHs detected. 2-methylnaphthalene was detected in the leaves of *E. senegalensis* with a concentration of 4.70 mg/Kg. Acenaphthene and fluorene with their respective concentrations of 0.35 mg/kg and 0.15 mg/kg were detected in the leaves of *F. umbellata* (Figure 5).

Plant samples from five different sampling clusters in this study site were taken through an acetone extraction and then subjected to silica gel column chromatography prior to reverse phase HPLC analysis. Concentrations were computed relative to concentrations of PAH standards. Results are means of $n = 2$.

PAHs in soil samples

Soil samples were taken from two different depths of 15-30 and 0-15 cm and used for the analysis in this study. The composite sampling of 3 different spots per location of 2 different depths, therefore the total number of soil sampled for this study was 60). The average concentrations of the various PAHs were calculated from all the sampling sites. Samples from Aboadze in the Shama district recorded seven PAHs from soil depth of 0-15 cm (Figure 6). The compounds were naphthalene (0.260 ± 0.580 mg/kg), acenaphthylene (3.360 ± 7.420 mg/kg), 2-methylnaphthalene (16.080 ± 35.870 mg/kg), acenaphthene (0.100 ± 0.220 mg/kg), fluorene (1.220 ± 1.800 mg/kg), phenanthrene (7.100 ± 15.870 mg/kg) and fluoranthene (7.300 ± 16.320 mg/kg).

Figure 7 shows mean concentrations of PAHs in soil samples from Dixcove in the Ahanta West District. The compounds (with concentrations at 0-15 cm and 15-30 cm, respectively) were as follows: acenaphthene (0.600 ± 1.475 and 1.100 ± 2.459 mg/kg), 1-methylnaphthalene (26.600 ± 59.500 and 105.800 ± 236.570 mg/kg), 2-methylnaphthalene (0.700 ± 1.565 and 0.220 ± 0.492 mg/kg), and anthracene (0.700 ± 1.565 and 0.86 ± 1.923 mg/kg). However, acenaphthylene (12.680 ± 28.350 mg/Kg), naphthalene (0.220 ± 0.490 mg/Kg), fluorene

(0.14 ± 0.313 mg/Kg), phenanthrene (2.82 ± 6.30 mg/Kg) and fluoranthene (8.626 ± 19.288 mg/kg) were detected at the depth of 15-30 cm. Only Pyrene (0.220 ± 0.492 mg/Kg) was found at depth of 0-15 cm.

Soil PAHs levels for Lower Axim in the Nzema East District are shown in Figure 8. Only 2-methylnaphthalene concentrations of 0.460 ± 1.020 and 0.66 ± 1.475 mg/kg at soil depth of 0-15 cm and 15-30 cm, respectively and pyrene at concentrations of 0.120 ± 0.268 and 0.180 ± 0.402 mg/Kg were detected. On the other hand, acenaphthene (0.420 ± 0.939 mg/kg) was detected only at depth of 15-30cm. PAHs were detected only at depths of between 0-15 cm in Half Assini of the Jomoro District. As shown in Figure 9, the PAHs were acenaphthene (0.160 ± 0.357 mg/kg), phenanthrene (1.580 ± 0.353 mg/kg), fluorene (0.100 ± 0.223 mg/kg), anthracene (0.200 ± 0.447 mg/kg) and fluoranthene (95.360 ± 21.322 mg/kg).

Soil samples from five different sampling clusters in this study site were taken through an acetone extraction and then subjected to silica gel column chromatography prior to reverse phase HPLC analysis. Concentrations were computed relative to concentrations of PAH standards. Blue bars indicate soil taken from 0-15cm. Red bars indicate soil taken from 15-30cm. Results are means $\pm$ SD. $N = 5$ (Figure 6-9).

Micronuclei in fish erythrocytes

Erythrocytes were observed using an optical microscope at $100\times$ magnification from ten different fields were counted and then averaged as a representative value before being normalized using equation 2.1. Only cells with intact cellular and nuclear membranes were scored. The mean and SD of micronuclei frequency per 1000 cells for each fish species was then computed from normalized micronuclei values from three fishes. The mean and standard deviation of micronucleated erythrocytes (MNEs) per 1000 cells observed in the different fish species from Atuabo is shown in Figure 10, and from Lower Axim is shown in Figure 11. Six fish species from Atuabo, namely *S. dumurili*, *Ca. chrysurus*, *S. sphyraena*, *E. dorsalis*, *S. dorsalis*, *C. hippos* were analysed for the presence of the micronuclei. Only the three species *S. sphyraena*, *Ca. chrysurus*, and *S. dorsalis* had micronuclei with their mean MNEs/1000 cells of 0.51 ± 0.88 , 0.13 ± 0.22 and 0.113 ± 0.22 , respectively (Figure 10). Figure 11 shows that in Lower Axim, all the six fish species, namely *D. angolensis*, *B. auritus*, *S. sphyraena*, *Ch. chrysurus*, *S. fiatola* and *P. typus* had mean MNEs/1000 cells of 0.41 ± 0.72 , 0.92 ± 1.59 , 6.98 ± 12.09 , 0.62 ± 1.07 , 1.36 ± 2.36 , 2.34 ± 4.06 , respectively. However, of the six fish species, namely *S. aurita*, *B. auritus*, *S. sphyraena*, *H. brasiliensis*, *P. incisus* and *A. monroviae* from Half Assini, only *B. auritus* recorded MNEs/1000 cells of 2.21 ± 3.38 (Table 2).

At Aboadze, *I. africana*, *B. auritus*, *P. incisus*, *D. congoensis*, *S. aurita*, *S. eba*, *P. prayensis*, *K. pelamis*, and *L. fulgens* recorded no MNEs. Likewise, fish species *P. quinquarius*, *G. decadactylus*, *P. senegalensis*, *B. auritus* and *I. africana* from New-Takoradi landing beach, Sekondi-Takoradi Metropolitan District had no MNEs. Moreover, the sampled fish species from Dixcove of the

Ahanta West District, e.g., *T. albacores*, *K. pelamis*, *T. lepturus*, and *T. alalunga*, recorded no MNEs.

Data of thin smears of peripheral blood presented as the mean frequency of micronuclei $\pm$ SD of $N = 3$. These data were obtained from three fish per six different fish species were stained with Giemsa and Maygrunwald solutions. ND means not detected. MN-Micronuclei, SD-Standard Deviation, MAX-Maximum, MIN-Minimum.

Table 2. Mean of micronuclei frequency/1000 cells from Half Assini in the Jomoro District of Western region of Ghana

Samples	Mean MN	SD	Max value	Min value
<i>Brachydeuterus auritus</i>	2.21	3.83	6.64	0.00
<i>Sphyraena sphyraena</i>	ND	ND	ND	ND
<i>Sardinella aurita</i>	ND	ND	ND	ND
<i>Hemiramphus brasiliensis</i>	ND	ND	ND	ND
<i>Pomadasys incisus</i>	ND	ND	ND	ND
<i>Acanthurus monroviae</i>	ND	ND	ND	ND

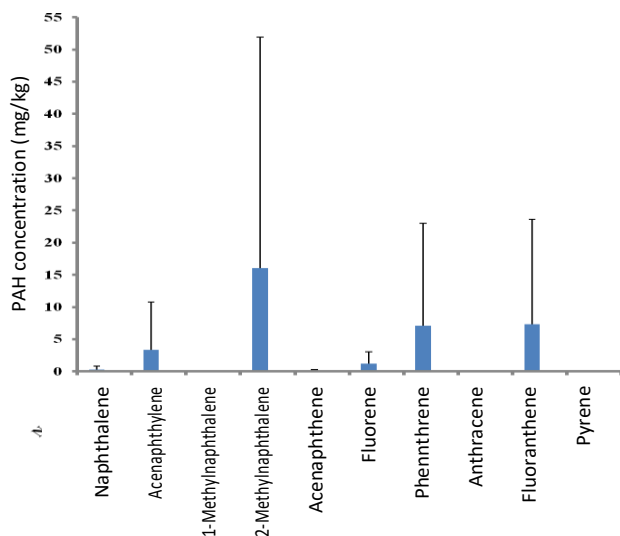


Figure 6. PAHs in soil samples from Aboadze in the Shama, Ghana

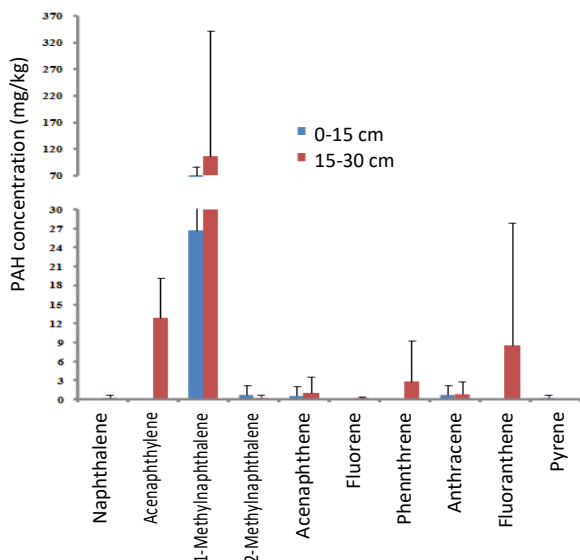


Figure 7. PAHs in soil samples from Dixcove in the Ahanta West District, Ghana

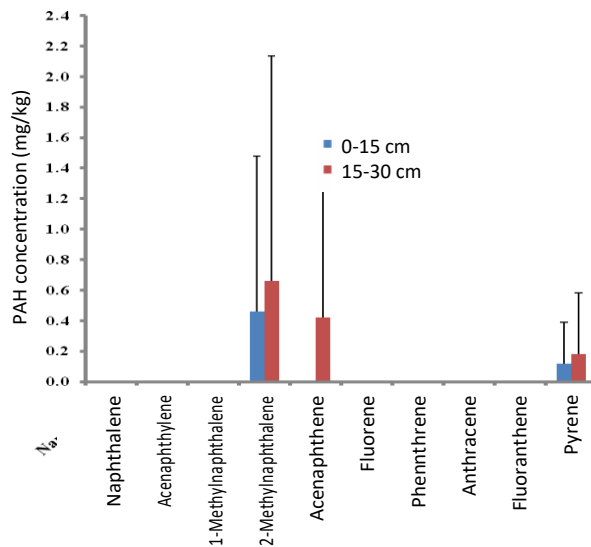


Figure 8. PAHs in soil samples from Lower Axim in the Nzema East District, Ghana

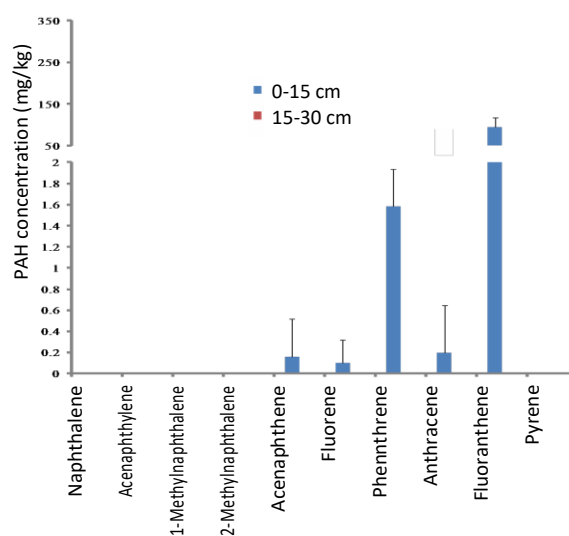


Figure 9. PAHs in soil samples from Half Assini in the Jomoro District, Ghana

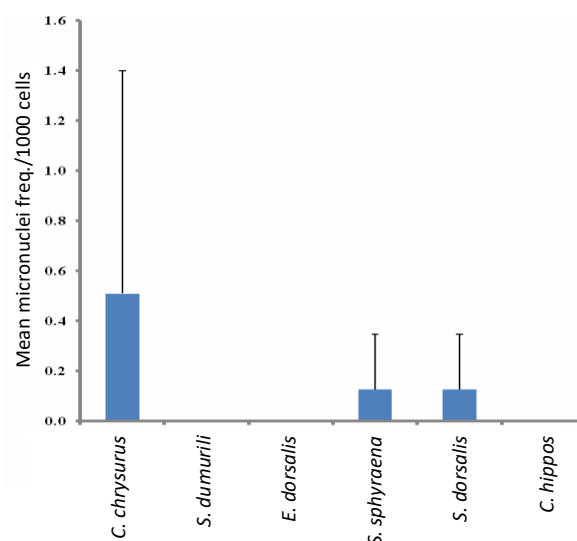


Figure 10. Mean micronuclei observed in fish species from Atuabo in Ellembele District, Ghana

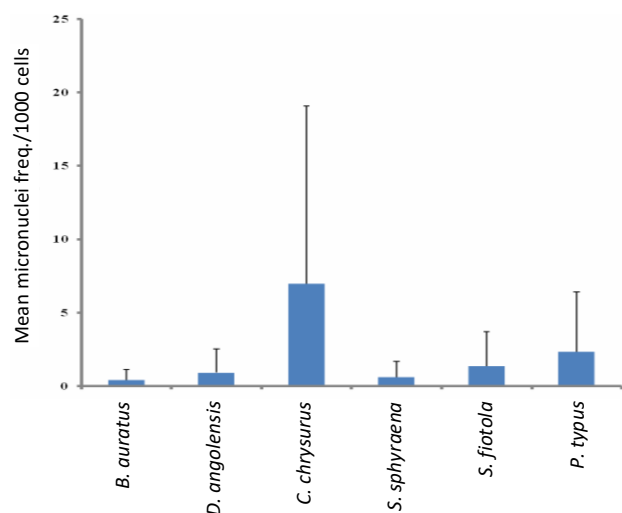


Figure 11. Mean micronuclei counts in different fish species from Lower Axim in Nzema East District, Ghana

Discussion

Many human activities contributed significantly to environmental pollution, e.g., oil drilling, industry, land reclamation, and continuous urbanization. The pollution released to the environment could have adverse consequences for human health when they are exposed to high levels of such toxicants from the air, animals, plants, and water bodies. These toxic substances can persist in the environment for some time because water bodies and land (all together with their fauna and flora), play a role in harboring, retaining and depositing these toxic substances (Li et al. 2008). In addition, some volatile toxic substances can readily contaminate atmospheric air. The most frequent contaminants surrounding oil drilling fields are oil spills, mineral oil, and polycyclic aromatic hydrocarbons (PAHs). Although there are hundreds of kinds of PAHs, the group of PAHs that pose health risks generally includes 1-methylnaphthalene, 2-methylnaphthalene, acenaphthene, naphthalene, acenaphthylene, fluorene, phenanthrene, fluoranthene, anthracene, pyrene, benz[a]anthracene, benz[a]fluoranthene, chrysene, and benz[k]fluoranthene. Here, we assessed the baseline assessment of biomarkers of pollution resulting from oil drilling of the Jubilee Oil Fields in the Western region of Ghana. The biomarkers of interest were the DNA adduct formation in fish samples. Accordingly, we also measured the presence of PAHs.

The presence of high levels of PAHs in fish tissues is suggestive of PAH contamination of coastal water from sources such as discharges from the crude oil drilling site, oil slicks, dumping of domestic wastes on the coast, accidental discharges of oil from ships and fishing boats, and smoke emissions from coastal industries (Neff and Burns 1996). Fishes are a good model of bioindicators for detecting the presence of pollutants in the aquatic environment because of their sensitivity to low concentrations of genotoxic substances (Al-Sabti and Metcalfe 1995). Fishes take up these toxicants and bio-concentrate them in organs such as gills, liver, and to a lesser extent, muscle tissues (Baussant et al. 2001).

Moreover, the hydrophobic nature of PAHs can accumulate in the fish's fatty tissues following ingestion (Bouloubassi et al. 2001). The distribution of PAHs in the water column depends on their lipophilic nature.

In most cases, high and low molecular weight PAHs from sediments from the water column by particles that tend to settle at the bottom of water bodies and volatilization (Neff and Burns 1996). Therefore, it would be expected that the tissue of pelagic fishes, which are surface water dwellers, would indicate the absence of high molecular weight PAHs or even if they are present, their concentration will be very low. In each of the six study sites selected, a number of fish species, pelagic and demersal, showed the presence of PAHs. Figure 2 indicates the presence of PAHs (naphthalene, acenaphthylene, 2-methylnaphthalene and acenaphthene) in demersal fish species *P. incises*. All of these substances are low molecular weight PAHs. Their uptake by this particular demersal species may be due to high PAHs concentrations in the sediments where this species dwells.

The predominance of lower molecular weight PAHs points to petrogenic sources; thereby, these low molecular weight PAHs in *P. incises* can be attributed to petrogenic sources, including crude oil drilling activities (Saha et al. 2009). Fluorene was the only PAH in the species *T. alalunga* caught from Dixcove. On the other hand, the absence of PAH in fish with micronuclei detected in their erythrocytes can be attributed to their being faster metabolizers of PAH than species such as *P. incises* and *T. alalunga* (Varanasi et al. 1989). Although we found very low levels of PAHs, such a low concentration of contaminants can trigger the genotoxicity effects of PAHs in fish and, by extension, in coastal dwellers (White 2002). However, PAHs concentrations above the threshold limit value of 30 µg/Kg are deemed toxic (US-EPA 2000). Fish consumption is part of a routine diet for people in Ghana as it constitutes a major source of animal protein (Asamoah et al. 2012). People living in the coastal region such as the study sites tend to consume large quantities of fish and hence could be at a greater risk of being exposed to PAHs and their toxic effects such as growth reduction (Reynaud and Deschaux 2006), malformations of the embryo, endocrine alteration, DNA damage and congenital disabilities (Choi et al. 2006).

Two major influx of PAHs ended up in the aquatic environment: the water movement, which contains dissolved and particulate constituents derived from watersheds, and atmospheric deposition both in precipitation and dry deposition from airsheds of the coastal ocean (Latimer and Zheng 2003). Additionally, oil spills and leaks during the transport and production of petroleum also account for significant levels of PAHs in coastal marine environments (Wang et al. 2007).

Currently, petroleum is the dominant energy source in the world, and it is expected to remain so over the next several decades (Kharaka and Dorsey 2005). Consequently, the continued release of PAHs into the environment, as the adverse effect of activities related to petroleum production, is inevitable. A baseline assessment of PAHs in water bodies used for domestic purposes in these study areas

revealed fluoranthene as the only PAHs detected significantly.

World Health Organization stated that the limit of concentrations of individual PAHs in water bodies is generally found to be 50 ng/L and hence any concentration above this level indicates rather toxic levels (WHO 2008). However, it must be pointed out that the study sites which closest to the drilling sites, harbor no manufacturing industries (they are mainly coastal fishing communities); thus, leaching and atmospheric deposition may directly account for the elevated levels of fluoranthene recorded, particularly in Dixcove. Leaching of PAH in water bodies is the primary route for contamination of water bodies because the compounds tend to be strongly adsorbed on soil organic matter (Oros et al. 2007). Previous reports stress out the elevated concentration of PAHs predominantly, fluoranthene, pyrene and phenanthrene in water bodies (WHO 2008), probably as a result of the adsorption of the compounds to air particulate matter, which is finely dispersed into the water during wet deposition and ultimately leaching into the water bodies. These PAHs are classified as heavy molecular weight PAH, thus they are easily leached as water solubility decreases with increasing molecular weight (Verweij et al. 2004). The baseline results in this study compare with the level of PAH contaminations in water bodies reported by England and Wales, where PAH levels are above the set standard of 0.2 µg/L in water bodies (Kirby et al. 1998). The factors contributing to the low levels of PAHs in water bodies, whether surface or groundwater, can be attributed to their hydrophobic nature, the ease with which they are taken up by aquatic organisms and their subsequent bioaccumulation and strong interaction with sedimentary organic carbon (Thorsen et al. 2004).

Plant leaves were also assessed for the existence of PAHs because leaves are considered important sinks for atmospheric PAHs and are involved in the periodic cycling of PAHs (Slaski et al. 2000; Zygmunt and Namiesnik 2003). Plants have demonstrated a good quantitative indicator of exposure to both gaseous and solid-phase PAHs in the environment (Korury et al. 1999; Zare-Maivan 2011). Plant leaves are the primary sinks of airborne PAH compounds, whereas absorption of PAHs from soils through the root system is minimal (De Nicola et al. 2011). The study sites detected PAHs in only two dominant species, *F. umbellata* and *E. senegalensis* from Lower Axim. Therefore, the plant species *E. senegalensis* and *F. umbellata* can be used as a good indicator for evaluating PAH pollution in the future. Accumulations of PAHs are aided by leaf surface area. Low molecular weight and volatile PAHs partition between the atmosphere and vegetation (Binet et al. 2000). At high ambient temperatures, the low molecular weight PAHs can re-volatilize into the atmosphere and affect the concentration of PAHs in plants. A wide variety of plant species have also been used as indicators to assess PAH pollution levels in heavily polluted industrial regions as well as for the identification of unknown points of emission (Baud-Grasset et al. 1993). These include *Lemna gibba*, *Brassica napus*, *Betula pendula* and *Morus rubra* (Baud-Grasset et

al. 1993; Duxbury et al. 1997; Vácha et al. 2010). Other reports have indicated a direct impact of soil and air pollution on PAHs content in plants (Salanitro et al. 1997). The very insignificant levels of PAHs detected in leaf samples suggest a very low concentration of PAHs in the atmosphere. This inference is supported by the fact that there were no manufacturing industries in the study sites, hence eliminating the inclusion of pyrogenic PAHs sources.

Some PAHs such as 2-methylnaphthalene, 1-methylnaphthalene, acenaphthene and anthracene were found in the different soil depths from Dixcove. However, acenaphthylene, naphthalene, fluorene, and phenanthrene were only found at depths of 15-30 cm, which is indicative of the leaching of these compounds to this depth, perhaps via petrogenic sources. Pyrene was the only high molecular weight PAH compound found in Dixcove in the 0-15 cm depth.

From the study site Lower Axim, acenaphthene, 2-methylnaphthalene, and pyrene were the only PAH compounds detected. Both acenaphthene and 2-methylnaphthalene are low molecular weight PAHs found at a depth of 15-30 cm. Pyrene, the only high molecular weight PAH found in Lower Axim, was also detected at both depths (surface and deep). At Half Assini, fluorene, acenaphthene, phenanthrene, fluoranthene, and anthracene were the PAHs detected, with all except fluoranthene being detected at a depth of 0-15 cm. Fluoranthene, the only high molecular weight PAH detected in this town, was found at both depths. Dixcove and Lower Axim are coastal towns close to the oil drilling site, thus these areas are at risk of PAH contamination. The predominant occupation in this community is fishing. Interestingly, all the PAHs found in Aboadze were low molecular weight PAHs detected at a depth of 0-15 cm. However, none of these low molecular weight PAHs had leached to a deeper depth, or the concentrations at this depth probably were too low to be detected. Aboadze is a coastal town with a thermal plant belonging to the Volta River Authority (VRA). Thermal plants burn diesel, a component of crude oil, to generate electricity. Thus, the PAH compounds in this study site could be partly attributed to the pyrogenic contaminants from energy generation via the thermal plant. Reasons such as high volatilization or dissolution could be assigned for the non-detection of very low molecular weight PAHs such as acenaphthylene, naphthalene, and acenaphthene on soil surfaces (depth of 0-15 cm). It is also possible that activities in the area have not yet generated any detectable PAHs levels. The results revealed fluoranthene and 1-methylnaphthalene as compounds with high concentrations in soil samples from Half Assini and Dixcove.

The frequency of over 15% micronucleus in an organism's peripheral erythrocytes indicates the presence of genotoxic agents such as PAHs in the environment (De Lemos et al. 2008). Thus, a baseline and periodic assessment of micronucleus are imperative to help regulate the release of these chemical pollutants into the aquatic environment and initiate the bioremediation process to curtail the potential toxic effects. Therefore, the micronucleus assay can be adopted for bio-monitoring

because it is a fast and sensitive indicator of structural alterations, DNA loss, and numerical chromosomal abnormalities (Galindo and Moreira 2009). In this environmental study, we employed different fish species from the communities bordering the oil drilling field to establish the baseline values for the biomarker in these sentinel organisms. No micronucleus was observed in fish species from these control sites (Aboadze, New-Takoradi and Dixcove) except the species *B. auritus* from the Jomoro District, where the mean frequency was (2.21 ± 3.83) MNE/1000. Similar studies carried out in Slovenia to establish baseline micronucleus frequency in fish species also gave low frequency (Al-Sabti and Metcalfe 1995; Bolognesi et al. 2006).

In addition, the low frequency of MNE and its variability in fish species determined in this study are similar to results found for the *Astyanax jacuhiensis* MNE baseline conducted in Brazil (De Lemos et al. 2008). However, there was no statistical significance between the mean micronuclei frequency of all the fish species analyzed, including the mean of *C. chrysurus* from Lower Axim, which showed the highest levels (6.98 ± 12.09) . Though our study's results suggest minimal contamination, continuous monitoring should be done since any change in the environment could be easily detected by an increase in these parameters. Nevertheless, micronuclei were observed in peripheral erythrocytes from fish species caught in the communities close to the drilling sites, albeit no statistical significance when analyzed statistically. Results from other studies indicate a very low range of MNE frequency and a large range of variability in the frequency of other fish species (Bolognesi et al. 2006). The variability could be brought about by differences in metabolic and pharmacokinetic factors (Bolognesi et al. 2006). The MNE biomarker is a less expensive, quick and sensitive means to detect the damage induced by petrochemical discharges. The association between nuclear anomalies' frequency and genotoxic agents' exposure is well established (Al-Sabti and Metcalfe 1995).

In conclusion, this study has provided baseline data for assessing the pollution of six districts nearby the drilling activities at the Jubilee oil fields in the Western region of Ghana. DNA adducts identified by the presence of micronuclei and PAH levels are useful markers for pollution. Therefore, the presence of micronuclei from fish erythrocytes and PAHs in water, soil, plant, and fish livers, especially at sites close to the oil fields, may indicate pollution level from the drilling activities over the past three years since its commencement. Generally, insignificant levels of DNA adducts and PAHs were detected in the samples analyzed.

REFERENCES

- Adewole GM, Adewale TM, Ufuoma E. 2010. Environmental aspect of oil and water-based drilling muds and cuttings from Dibi and Ewan off-shore wells in the Niger Delta, Nigeria. *Environ Sci Technol* 4 (5): 284-292.
- Al-Sabti K, Metcalfe CD. 1995. Fish micronuclei for assessing genotoxicity in water. *Mutat Res Genet Toxicol* 343 (3): 121-135. DOI: 10.1016/0165-1218(95)90078-0.
- Asamoah EK, Ewusie Nunoo FK, Osei-Asare YB, Addo S, Sumaila UR. 2012. A production function analysis of pond aquaculture in southern Ghana. *Aquac Econ Manag* 16 (3): 183-201. DOI: 10.1080/13657305.2012.704616.
- Baud-Grasset F, Baud-Grasset S, Safferman S. 1993. Evaluation of the bioremediation of a contaminated soil with phytotoxicity tests. *Chemosphere* 26: 1365-1374. DOI: 10.1016/0045-6535(93)90187-A.
- Baessant T, Sanni S, Jonsson G, Skadsheim A, Børseth JF. 2001. Bioaccumulation of polycyclic aromatic compounds: Bioconcentration in two marine species and in semipermeable membrane devices during chronic exposure to dispersed crude oil. *Environ Toxicol Chem* 20 (6): 1175-1184. DOI: 10.1002/etc.5620200606.
- Binet P, Portal JM, Leyval C. 2000. Fate of polycyclic aromatic hydrocarbons (PAH) in the rhizosphere and mycorrhizosphere of rye grass. *Plant and Soil* 227: 207-213. DOI: 10.1023/A:1026587418611.
- Bolognesi C, Perrone E, Roggieri P, Pampanin DM, Sciuotto A. 2006. Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. *Aquat Toxicol* 78 (1): 93-98. DOI: 10.1016/j.aquatox.2006.02.015.
- Bouloubassi I, Fillaux, J, Saliot A. 2001. Hydrocarbons in surface sediments from the Changjiang (Yangtze River) Estuary, East China Sea. *Mar Pollut Bull* 42 (12): 1335-1346. DOI: 10.1016/S0025-326X(01)00149-7.
- Choi H, Jedrychowski W, Spengler J, Camann DE, Whyatt R, Rauh V, Perera FP. 2006. International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. *Environ Health Perspect* 114 (11): 1744-1750. DOI: 10.1289/ehp.8982.
- De Lemos CT, Iraújo FDA, de Oliveira NCD, de Souza GD, Fachel JMG. 2008. Biomonitoring of genotoxicity using micronuclei assay in native population of *Astyanax jacuhiensis* (Characiformes: Characidae) at sites under petrochemical influence. *Sci Total Environ* 406 (1-2): 337-43. DOI: 10.1016/j.scitotenv.2008.07.006.
- De Nicola F, Lancellotti C, Prati M, Maisto G, Alfani A. 2011. Biomonitoring of PAHs by using *Quercus ilex* leaves: Source diagnostic and toxicity assessment. *Atmos Environ* 45 (7): 1428-1433. DOI: 10.1016/j.atmosenv.2010.12.022.
- Denton GRW, Concepcion LP, Wood HR, Eflin VS, Pangelinan GT. 1999. Heavy Metals, Pcb's and Pch's in Marine Organisms from Four Harbour Locations on Guam. Technical Report No. 87, Water and Environmental Research Institute of the Western Pacific, University of Guam Mangilao.
- Devold H. 2013. Oil and Gas Production Handbook An Introduction to Oil and Gas Production, Transport, Refining and Petrochemical Industry. ABB Oil and Gas.
- Duxbury CL, Dixon DG, Greenberg BM. 1997. The effects of simulated solar radiation on the bioaccumulation of polycyclic aromatic hydrocarbons by the duckweed *Lemna gibba*. *Environ Toxicol Chem* 16: 1739-1748. DOI: 10.1002/etc.5620160824.
- Ecobank. 2014. Ecobank Country Profile: Ghana, An Economic Outlook Report. UK.
- Feng X, Pisula W, Müllen K. 2009. Large polycyclic aromatic hydrocarbons: Synthesis and discotic organization. *Pure Appl Chem* 81 (12): 2203-2224. DOI: 10.1351/PAC-CON-09-07-07.
- Galindo TP, Moreira LM. 2009. Evaluation of genotoxicity using the micronucleus assay and nuclear abnormalities in the tropical sea fish *Bathygobius soporator*. *Genet Mol Biol* 32 (2): 394-398. DOI: 0.1590/S1415-47572009000200029.
- German Federal Environment Agency. 2012. Polycyclic Aromatic Hydrocarbons: Harmful to the Environment! Toxic! Inevitable. Dessau-Roßlau, Germany.
- Ghana Statistical Service. 2012. Population and Housing Census. Summary Report of Final Results. Ghana Statistical Service . Sakoa Press Ltd., Accra.
- Kharaka YK, Dorsey NS. 2005. Environmental issues of petroleum exploration and production: Introduction. *Environ Geosci* 12 (2): 61-63. DOI: 10.1306/eg.intro0605020205.
- Kirby MF, Blackburn MA, Thain JE, Waldo MJ. 1998. Assessment of water quality in estuarine and coastal waters of England and Wales using a contaminant concentration technique. *Mar Pollut Bull* 36 (8): 631-642. DOI: 10.1016/S0025-326X(98)00051-4.
- Korury S, Teimori M, Khoshnevis M, Salahi P, Matiniazadeh M, Moraghebi F, Maghooli F, Shirvani A. 1999. Evaluation of pollution damage from the Persian Gulf war on mangroves and coastal vegetation. *Pajouhesh va Sazandegi J* 43 (12): 102-107.

- Latimer JS, Zheng J. 2003. The sources, transport, and fate of PAH in the marine environment. In: Douben PT (eds). PAHs: An Ecotoxicological Perspective. John Wiley and Sons Ltd., New York.
- Li H, Wei XW, Yu XL. 2008. Heavy metals and PAHs in sewage sludge from twelve wastewater treatment plants in Zhejiang Province. Biomed Environ Sci 21: 345-352. DOI: 10.1016/S0895-3988(08)60053-7.
- Lyons RA, Temple JM, Evans D, Fone DL, Palmer SR. 1999. Acute health effects of the Sea Empress oil spill. J Epidemiol Commun Health 53 (5): 306-310. DOI: 10.1136/jech.53.5.306.
- Madlener R, Sunak Y. 2011. Impacts of urbanization on urban structures and energy demand: What can we learn for urban energy planning and urbanization management? Sustain Cities and Soc 1 (1): 45-53. DOI: 0.1016/j.scs.2010.08.006.
- Mishra M, Das S. 2017. Profiling marine bacterial dioxygenase gene involved in Polycyclic Aromatic Hydrocarbon degradation. Ocean Life 1: 32-40. DOI: 10.13057/oceanlife/o010206.
- Neff JM, Burns WA. 1996. Estimation of PAH concentrations in the water column based on tissue residues in mussels and salmon: an equilibrium partitioning approach. Environ Toxicol Chem 15 (12): 2240-2253. DOI: 10.1002/etc.5620151218.
- Oros DR, Ross JRM, Spies RB, Mumley T. 2007. Polycyclic aromatic hydrocarbon (PAH) contamination in San Francisco Bay: A 10-year retrospective of monitoring in an urbanized estuary. Environ Res 105 (1): 101-118. DOI: 10.1016/j.envres.2006.10.007.
- Pampanin DM, Sydnese MO. 2013. Polycyclic aromatic hydrocarbons a constituent of petroleum: Presence and influence in the aquatic environment. Intech Open. DOI: 10.5772/48176.
- Reynaud, S, Deschaux P. 2006. The effects of polycyclic aromatic hydrocarbons on the immune system of fish: A review. Aquat Toxicol 77 (2): 229-238. DOI: 10.1016/j.aquatox.2005.10.018.
- Saha M, Togo A, Mizukawa K, Murakami M, Takada H, Zakaria MP, Tana TS. 2009. Sources of sedimentary PAHs in tropical Asian waters: Differentiation between pyrogenic and petrogenic sources by alkyl homolog abundance. Mar Pollut Bull 58 (2): 189-200. DOI: 10.1016/j.marpolbul.2008.04.049.
- Salanitro JP, Dorn PB, Huesemann MH, Moore KO, Rhodes IA, Jackson LMR, Vipond TE, Western MM, Wisniewski HL. 1997. Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. Environ Sci Technol 31: 1769-1776. DOI: 10.1021/es960793i.
- Slaski JJ, Archambault DJ, Li X. 2000. Evaluation of polycyclic aromatic hydrocarbon (PAH) accumulation in plants. The potential use of PAH accumulation as a marker of exposure to air emissions from oil and gas flares. Report prepared for the Air Research Users Group, Alberta Environment, Edmonton, Alberta. DOI: 10.5962/bhl.title.115659.
- Thorsen WA, Cope WG, Shea D. 2004. Bioavailability of PAHs: Effects of soot carbon and PAH source. Environ Sci Technol 38 (7): 2029-2037. DOI: 10.1021/es0306056.
- U.S. Environmental Protection Agency (US-EPA). 2000. Risk Based Concentration Table. United States Environmental Protection Agency, Philadelphia, PA, Washington DC.
- Vácha R, Čechmánková J, Skála J. 2010. Polycyclic aromatic hydrocarbons in soil and selected plants. Plant Soil Environ 56 (9): 434-443. DOI: 10.17221/7/2010-PSE.
- Varanasi U, Stein JE, Nishimoto M. 1989. Biotransformation and disposition of PAH in fish In: Varanasi U (eds). Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment. CRC Press, Boca Raton, Florida USA.
- Verweij F, Booi K, Satumalay K, Van Der Molen N, Van Der Oost R. 2004. Assessment of bioavailable PAH, PCB and OCP concentrations in water, using semipermeable membrane devices (SPMDs), sediments and caged carp. Chemosphere 54 (11): 1675-1689. DOI: 10.1016/j.chemosphere.2003.10.002.
- Wang L, Lee FSC, Wang X, Yin Y, Li J. 2007. Chemical characteristics and source implications of petroleum hydrocarbon contaminants in the sediments near major drainage outfalls along the coastal of Laizhou Bay, Bohai Sea, China. Environ Monit Assess 125 (1-3): 229-237. DOI: 10.1007/s10661-006-9514-0.
- Wang Z, Stout SA, Fingas M. 2006. Forensic fingerprinting of biomarkers for oil spill characterization and source identification. Environ. Forensics 7 (2): 105-146. DOI: 10.1080/15275920600667104.
- White PA. 2002. The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. Mutat Res Genet Toxicol Environ Mutagen 515 (1-2): 85-98. DOI: 10.1016/s1383-5718(02)00017-7.
- World Bank. 2009. Economy-Wide Impact of Oil Discovery in Ghana Report No.47321-GH.2-4.
- World Health Organization (WHO). 2008. Guidelines for drinking-water quality: incorporating 1st and 2nd addenda. WHO chronicle. Vol. 38.
- Zare-Maivan H. 2011. Polycyclic Aromatic Hydrocarbons (PAHs) in Plants of Shadegan Wetland: *Halocnemum strobilaceum* and *Suaeda maritima*. J Persian Gulf 2 (3): 37-41.
- Zygmunt B, Namiesnik J. 2003. Preparation of samples of plant material for chromatographic analysis. J Chromatogr Sci 41 (3): 109-116. DOI: 10.1093/chromsci/41.3.109.