BASELINE ASSESSMENT OF BIOMARKERS FOR CRUDE OIL POLLUTANTS: DNA ADDUCTS IN FISH AND POLYCYCLIC AROMATIC HYDROCARBONS IN SOME ENVIRONMENTAL SAMPLES IN THE WESTERN COAST OF GHANA

BY

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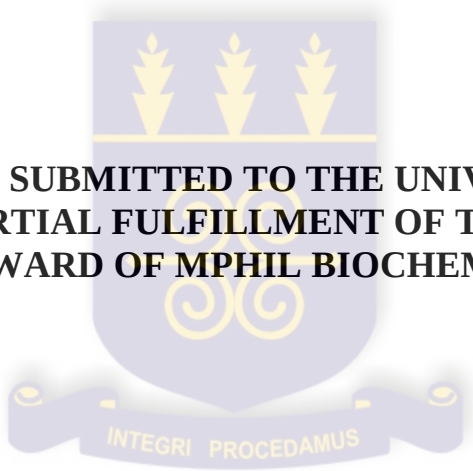

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DECLARATION

The experimental work presented in this thesis was done by me, Ebenezer-Osei-Yeboah, at the Clinical Pathology department of Noguchi Memorial Institute for Medical Research (NMIMR) under the supervision of Dr. Regina Appiah-Opong and Dr .Mark Ofosuhene (both of NMIMR) and that no part of it has been submitted anywhere else for any other purpose.

All references to the work of other persons or bodies have been duly acknowledged.

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I would like to extend my appreciation to all members of staff of Biochemistry, Cell and Molecular Biology department, University of Ghana.

DEDICATION

To Jehovah God almighty, my parents and sisters.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATION

ACGIH	American Conference of Governmental Industrial Hygienists
AETC	African and Eastern Trade Corporation
AhR	Arylhydrocarbon Receptor
ARNT	Arylhydrocarbon Receptor Nuclear Translocator
ASE	Accelerated Solvent Extraction
ATSDR	Agency for Toxic Substances and Disease Registry
BOPD	Barrels of Oil Per Day
CCEH	Centre for Children's Environmental Health
CYP	Cytochrome P450
DCM	Dichloromethane
EPA	Environmental Protection Agency
FAO	Food and Agricultural Organization
FPSO	Floating Production Storage and Offloading
GNPC	Ghana National Petroleum Corporation
HPLC	High Pressure Liquid Chromatography
Hsp90	Heat Shock Proteins 90

LI	Legislative Instrument
MAE	Microwave Assisted Extraction
MCL	Maximum Contaminants Level
MNE	Micronucleated Erythrocytes
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupation Safety and Health Administration
PAHs	Polycyclic Aromatic Hydrocarbon
PHWE	Pressurized Hot Water Extraction
PLE	Pressurized Liquid Extraction
PNDC	Provisional National Defense Council
REL	Recommended Exposure Limit
ROS	Reactive Oxygen Species
SD	Standard Deviation
SFE	Supercritical Fluid Extraction
SFP	Societe Francaise De Petrole
SPE	Solid Phase Extraction
USD	United States Dollars

USEPA	United State Environmental Protection Agency
UV	Ultra Violet
WAOFCO	West Africa Oil and Fuel Company
WHO	World Health Organization

ABSTRACT

The industrialization of our society, including drilling for oil, mining of coal and minerals has led to increasing production of xenobiotic and natural chemical substances. Continual releases of polycyclic aromatic hydrocarbons (PAHs) from mining and drilling activities leads to their deposition in coastal environments and ultimately bioaccumulation in plants and animals, creating the danger of toxicity. PAH are deemed to be carcinogenic, mutagenic and teratogenic. In Ghana, oil drilling is ongoing at the Jubilee oil fields in the Western region, however, onshore baseline environmental assessment of pollutants such as PAHs have not yet been performed.

In this study, an environmental assessment of some communities bordering the oil drilling fields was performed to establish (1) levels of fish DNA adduct formation of fishes, (2) levels of polycyclic aromatic hydrocarbons in soil, plants, water and fishes. Examination of blood smears of several fish species showed the presence of micronuclei in fish from three of the study areas although their mean micronucleated frequencies were low and below the threshold frequency of 15%. There was no statistical difference between their mean frequencies upon one way ANOVA analysis with a p value < 0.05 except the mean of the fish *Chloroscombrus chrysurus* which showed a significant difference. Reverse phase HPLC analysis of fish, water, plant and soil samples collected from six study sites were done. Only two fish species *Pomadasys incises* at Aboadze and *Thunnus alalunga* from Dixcove recorded four and one PAH compounds respectively with concentrations above the maximum contaminant levels of 30 µg/Kg set by the

USEPA. Mean concentration of PAHs in water samples were in the concentration range 1.4 to 1255 µg/L in water samples from two of the four study areas where water was found. 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* and *Ficus umbellata* recorded the presence of PAHs with concentrations in the range of 0.15-4.70 mg/Kg. Soil samples were collected from 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 were similar with 2–3 ring PAHs being dominant which is suggestive of petrogenic source.

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CHAPTER ONE

1.0 INTRODUCTION AND LITERAURE REVIEW

1.1 INTRODUCTION

1.1.1 Background

Crude oil is commonly classified as one of the highest if not the highest valued resource a country can possess, as it plays a very important role in modern society. This is basically due to the fact that oil is currently the dominant energy source and it is expected to remain so over the next several decades (Wang *et al*., 2006). Burgeoning populations worldwide and increasing number of countries becoming more industrialized, places high demand on energy and crude oil and its products are the most realistic source of meeting this demand (Madlener and Sunak, 2011).

Ghana recently discovered crude oil in commercial quantities off the shores of its Western Atlantic Coast. This discovery was made on the back of a century of exploration activities with input of huge amount of financial resources (Ecobank, 2014). However, Ghana had to wait three decades to achieve the much awaited commercially significant oil discovery in 2007 by the Jubilee partners; so called because the discovery coincided with jubilee anniversary since Ghana attained independence from the UK in 1957. Thus the first well was christened Jubilee and the Jubilee partners include Tullow Ghana limited, Sabre Oil and Gas Limited and Kosmos Energy Ghana. Reserves of the Jubilee fields as of October, 2009 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 averaged 102,503 barrels of oil per day (bopd).

Notwithstanding the seemingly crucial contribution of revenue from crude oil export to national development, the toxic composition of crude oil cannot be ignored as crude oil is a complex mixture of hydrocarbons containing more than 17,000 compounds (Pampanin and Sydnes, 2013). Among the constituents of crude oil is a group of substances called polycyclic aromatic hydrocarbons (PAHs) and as implicit from their name; PAHs are constituted from two to eight conjugated ring systems and consequently exhibit all the chemical properties of aromatic compounds. They can have a range of substituents such as alkyl, nitro, 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). They can be formed naturally by low-temperature, high-pressure reactions of natural organic matter and in this way constitute a significant fraction of petroleum hydrocarbons (Latimer and Zheng, 2003).

The PAHs that are present in the marine environment in relevant concentrations are divided into two groups according to their origin, namely pyrogenic and petrogenic (Pampanin and Sydnes, 2013). The petrogenic PAH are derived from oil and drilling activities such as oil disasters, spills, and effluence from industrial sites, oil refineries, and most significantly traffic exhaust emissions, while the pyrogenic PAH are derived

from forest fires, volcanic eruptions, and incineration. Generally pyrogenic PAHs are composed of larger ring systems usually above 6 rings while petrogenic PAHs are composed of 2 to 6 rings. The PAHs that pollute the coastal areas and its water bodies primarily come from effluent released by industries, runoff from roads, the smelter industries and oil spills. Also produced water that is generated from drilling routine activities can be a source of PAH pollution in the marine environment.

Oil seeps are natural springs where liquid and gaseous hydrocarbons trickle out of the ground and these are found scattered all over the globe with a higher concentration in certain regions of the world (Devold, 2013). Numerous times the presence of a natural oil seep has resulted in the discovery of oil reservoirs that are large enough for commercial oil production (Devold, 2013).

In the course of oil drilling, various chemicals and materials are released into the environment, e.g. drill cuttings, drilling mud, oil and chemicals injected into them 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 have been associated with adverse environmental and public health effects (Lyons *et al*., 1999). Their resultant adverse effects include the dead and moribund marine animals as well as the oil coating of shorelines and other water bodies, and PAH pollution of the environment (particularly coastal waters, plants and other biological organisms). The percentage of PAH in crude oil ranges between 0.2% and 7% (German Federal Environment Agency, 2012). Many PAHs are carcinogenic, mutagenic or teratogenic. Some PAHs are bioaccumulative, persistent and toxic to humans and other

organisms. Substances that combine these three characteristics represent a particular level of concern to public health and the environment (German Federal Environment Agency, 2012). Therefore, it is necessary to track the levels of PAH in coastal zones and in this regard biomarkers become all the more crucial as they serve as indicators of PAH pollution. Fishes are suitable model organisms to use as environmental genotox bio-indicator organisms; this is largely due to their role in the aquatic trophic chain and because of their sensitivity to low concentrations of genotoxic substances (Al-Sabti and Metcalfe, 1995). Genotoxic effects of PAH on fish can be evaluated by a number of assays, which includes micronuclei analysis in peripheral blood erythrocytes (Al-Sabti and Metcalfe, 1995). This stems from the fact that the micronucleus assay is a quick, sensitive and reliable assay to determine damage to the DNA. Micronuclei can originate both from acentric fragments resulting from chromosomal breaks which are not incorporated into the main nucleus and from whole chromosomes delayed during cellular division anaphase (De Lemos *et al*., 2008). Genotoxicity results from damage to DNA and one of the ways that this can happen is through the formation of DNA adducts. DNA adduct formation refers to the covalent binding of a highly electrophilic chemicals to DNA thus altering its structure and rendering it unable to undergo processes of replication, transcription, and repair. If the proper DNA conformation is not restored by repair mechanisms and adducts persists, these alterations may cause mutations and ultimately result in cancer development.

1.1.2 Problem Statement

The coastal environment where offshore drilling is carried out is usually inundated with chemical pollutants through the activities of man in various ways including oil drilling.

In the course of oil drilling, various chemicals and materials are released into the environment, e.g. drill cuttings, drilling mud, oil and chemicals injected into them to control corrosion or assist the separation of oil from water as well as general industrial waste. Spillage or discharge of these wastes into the sea and/or land have been associated with adverse environmental and public health effects (Okoh, 2006). The resultant adverse effects include the dead and moribund marine animals as well as the oil coating of shorelines and other water bodies (Adewole *et al.*, 2010), PAH pollution of the environment; particularly coastal waters, plants and other biological organisms. Once these PAHs are present in an environment, they accumulate in biological organisms and ultimately get amplified along food chains and they remain a potential threat for many years.

The PAHs resulting from oil drilling activities constitute considerable health threats to humans, animals and plants due to their mutagenic and carcinogenic abilities. The US EPA has identified 16 of the PAHs to be very toxic and these include: acenaphthylene, acenaphthene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, chrysene, dibenzo[ah]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene, and pyrene (Khadhar *et al.*, 2010). Knowledge of the aforementioned challenges of PAH exposure has by convention necessitated the development of standards and preventive measures for public and community health protection requiring referential environmental assessment and further comparative monitoring for critical levels of crude oil toxicants. Thus, several bioassays have been developed which provide a direct indication of the

level of exposure of humans, animals, plants and the environment as a whole to potentially toxic agents. For example, assessment of micronuclei in fish and concurrent analysis of PAHs in fish muscles and other samples such as plant leaves, soil and water will serve as a useful marker for exposure of coastal communities bordering the drilling site and people living in these areas to environmental pollutants such as PAHs.

1.1.3 Justification

With the discovery of crude oil in commercial quantities in 2007 and the subsequent commencement of drilling activities in the year 2010 at Tano/Cape Three Points, also known as the Jubilee Oil field, discovered 60 Km offshore in the Western region of Ghana, comprehensive environmental baseline studies are required by law (Environmental Protection Agency Act 1994, Act 490, parts 1&II) and Environmental Assessment Regulations 1999, LI 1652) to be carried out. This is to ensure that environmental quality control programme that requires prior environmental impact assessments of all new investments that would be deemed to affect the quality of the environment have been instituted and implemented.

In this regard a company, TDI-Brooks International in collaboration with Tullow-Ghana Oil Company, has performed a baseline assessment of the Jubilee oil field from 2008 to 2009. However, information on the reported assessment was on the offshore marine environment, but no onshore baseline data on pollutants such as PAH was provided. The availability of environmental assessment data on the localized communities is most critical for future comparative referencing and petrochemical monitoring towards decision making and emergency response in case of

future spillages. Thus, there is need for an onshore environmental assessment of communities in closest proximities to the Jubilee oil field in the Western Region of Ghana.

1.1.4 Aim

The aim of the study is to perform baseline onshore environmental impact assessment of communities bordering the Jubilee Oil field in the Western Region of Ghana

1.1.5 Specific Objectives

1. To assess hepatic DNA adduct formation in selected fish species.
2. To determine the PAH levels in fish harvested in the communities.
3. To assess the levels of PAH in water, plants and soil samples from the selected communities.

1.2 LITERATURE REVIEW

1.2.1 History of Crude Oil Exploration in Ghana

Explorative activities for oil and gas began in Ghana as far back as 1896 with some discoveries albeit in insignificant commercial quantities (Sakyi *et al*., 2005). These minor discoveries were made by the West Africa Oil and Fuel Company (WAOFCO) (Osei-Bonsu, 2011). WAOFCO's exploration activities were largely concentrated in the rich onshore Tano fields in Ghana's Western Region**.** A French petrochemical company, Societe Francaise de Petrole (SFP) in the period between 1909 and 1913, was engaged in explorative activities with the aim of finding oil in commercial quantities in the Gold Coast**.** Ultimately, SFP managed to drill a total of six (6) wells from which its first well,

the SFP-1 had oil reserve to a depth of 10-17 meters and produced 7 barrels of oil per day (bopd) for a brief period (Osei- Bonsu, 2011). After the brief drilling activities of SPF, African and Eastern Trade Corporation (AETC) from 1923 to 1925 also ventured into prospecting for oil in the Tano Basin even though other companies had little success in drilling oil**.** Gulf Oil Company (GOC) also ventured into drilling activities on the Onshore Tano basin with the aim of discovering oil in commercial quantities but had little success (Sutherland, 2008).

Post independence drilling activities saw a significant shift from onshore exploration activities to prospecting for oil offshore. This change was orchestrated by some Russian and Romanian geoscientists in 1960. These scientists conducted their explorative activities for petroleum resources in the Volta and Accra/Keta basins. Their exploratory activities and their resulting geophysical data however led to a very important operational shift; a shift from onshore to offshore shallow waters exploration (GNPC, 2009).

From 1970 to 1985, a number of oil companies invested in explorative and drilling activities in Ghana. The most significant discovery within this period was Signal oil's discovery in 1970 (Osei- Bonsu, 2011).

Under the Provisional National Defense Council (PNDC) regime, the petroleum department was re-organised to form the Ghana National Petroleum Corporation (GNPC) backed by Ghana's first petroleum law, Ghana National Petroleum Corporation (GNPC) Law, 1983 which was passed in 1983. Under section 26 of that Law, suitable staff of the Petroleum Department were transferred to form the core of that infant GNPC (GNPC,

2009). Another law, PNDCL 64, enacted in 1984 to provide statutory and legal framework brought about progress in exploration and production activities. Under this law, GNPC's corporate mission was to promote, explore and develop the hydrocarbon resources of the nation through lean, efficient and technology-driven investments so as to enhance the economic development of Ghana (GNPC, 2009).

The period between 2000 and 2007 saw re-organization of GNPC to specifically focus on exploration in deepwater stratigraphic traps. This saw the recruitment of companies with the requisite technical knowhow in this endeavour and in the year 2004 Kosmos Energy was engaged to prospect for oil in the Deep Water Cape Three Points block. Subsequently in 2006, the Ghana government signed an agreement with Tullow Oil to prospect the Deep and Shallow Water Tano blocks (Sutherland, 2008). These concerted and well focused efforts yielded the much awaited success in terms of discovering oil in commercial quantities after a century of investment.

1.2.2 Importance of Oil in the World

Crude oil is commonly classified as one of the highest resources a country can extract due to the fact that it is a vital ingredient in achieving sustained growth of many nations and Ghana is not an exception. The impact of the oil on Ghana's economy cannot be overemphasized; since the start of oil production, there have been a huge jump in Ghana's revenue. The government of Ghana has received 2.167 billion United States dollars as petroleum revenue from the commercial production and export of oil from 2011 to 2014 (Koranteng, 2014).

Crude oil is the main source of energy for the productive sectors of the Ghanaian economy. It accounted for about 96.7%, 52% and 92% of energy consumption in the agricultural sector, formal manufacturing sector and transport sector, respectively (Armah, 2003). "The 2013 performance of the Industry Sector was mainly on account of a 37.5 percent growth in petroleum activities, which fed into a 17.6 percent growth in the Mining and quarrying sub-sector, up from 5.0 percent in 2012" (Tekper,2014). In addition to contributing to increased growth rate, oil sector has resulted in expansion of the gross domestic product; another key macroeconomic indicator**.** Largely the GDP has expanded from 39 billion United States dollars (USD) in 2011 to 40 billion USD in 2012, 46 billion USD in 2013 and 50 billion USD in 2014**.**

1.2.3 Crude Oil Production

Crude oil production begins with drilling a well irrespective of whether the well location is offshore or onshore. Offshore drilling is done with the aid of a wide range of different structures, depending on size and water depth. Oil and gas production at the jubilee oil fields in Cape Three Points is done on a floating production storage and offloading (FPSO) structure. An FPSO is normally a tanker type hull or barge, often converted from an existing crude oil tanker or newly constructed one (Devold, 2013). Usually FPSO are employed for deep sea depth drilling. It has its wellheads or sub-sea risers from the sea bottom located on a central or bow-mounted turret which allows the ship to rotate freely to point into wind, waves or current (Devold, 2013).

Drilling of a well is the first in a series of a number of events that involve and release toxic chemicals that can contaminate air, water, and land (Currie and Isaacs, 2005).

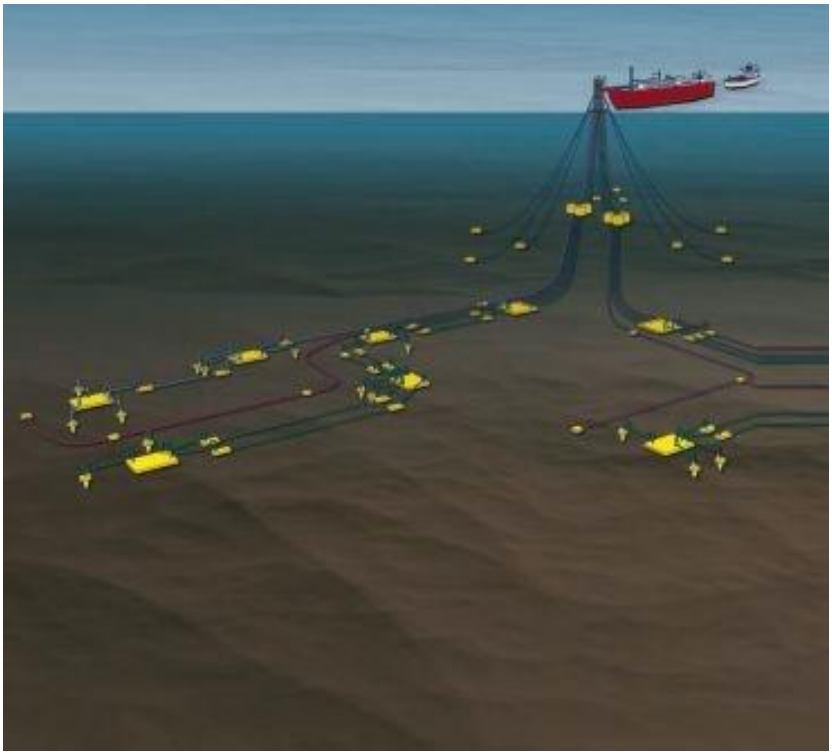

Figure 1. 1 Schematic representations of FPSO subsea risers and well heads on the ocean floor. (Source: www.Ghanaoilwatch.org)

The drilling process involves the use of toxic substances such as pipe dope to reduce friction, and drilling fluid, or hydraulic fluids to maintain well pressure and are subsequently disposed of as waste or spilled into the marine environment (Mall *et al*., 2007). Once drilling is done, a well begins producing oil, gas, or both along with large amounts of a fluid known as produced water, which contains oil and toxic substances (Mall *et al*., 2007)**.** The next stage in the production process after oil or gas has been pumped out of the well is the separation stage**.** This stage involves separating the various constituents of the raw product pumped from the well and it usually includes separating dirt or sand from crude oil, crude oil (petroleum) from natural gas and natural gas from dirt, sand, water vapor, or other gasses such as carbon dioxide, hydrogen sulfide,

propane, and butane (Devold, 2013). After separation has been done, the oil separated from the natural gas is then stored or offloaded to tanker for further processing. The wastes generated are transferred to a disposal pit and this can pose serious health threat to people living close to these disposal pits as the wastes are also sources of pollutants (Mall *et al.*, 2007).

1.2.4 Pathway for Contaminations

Offshore drilling routine operations inundate the marine environment with toxic wastes and chemicals that result in environmental pollution. The liquid and solid wastes associated with petroleum hydrocarbon operations include wastes derived from drilling activities, those derived from maintenance of machines and equipment and those derived from life on drilling platform (Okoh, 2006). The drilling stage of crude oil production is credited with large proportion of release of the toxic waste and chemicals which include tens of thousands of gallons of waste drilling muds (materials used to lubricate drill bits and maintain pressure), drill cuttings, produced water, cement slurry residue and oil cushions (Adewole *et al.,* 2010**).** Drill cuttings are the soil, rock fragments, and broken up material that are bought from a well and may contain an amount of fluid that results from a drilling process (Ball *et al.*, 2012). Even though drilling fluids are recovered and reused again some find their way into the marine environment by coating and adhering to the drill cuttings (Breuer *et al*., 1999). These toxic wastes from drilling process are composed of aliphatic hydrocarbons, polycyclic aromatic hydrocarbon (PAH) and heavy metals such as arsenic, cadmium, chromium, lead and mercury (Alimi *et al*., 2003).

Owing to their sizes (2 – 5 mm), almost all the particles of the drill cuttings disposed in the sea are deposited below drilling platforms in a thin uniform layer (Davies *et al*., 1984). The disposed well cuttings becomes a problem to the environments, offshore or onshore, when they become impregnated with oil during drilling as many of the additives components in the disposed drilling mud are toxic to the environment (Davies *et al*., 1984). The oil based drill cuttings and mud have been found to be associated with both saturated (60%) and unsaturated (40%) hydrocarbon which when bioaccumulated in the body of the fish can become carcinogenic on consumption (Trannum *et al*., 2010).

The water that is brought up from a given well along with oil and gas is referred to as "produced water"(Fakhru'l-Razi *et al*., 2009). This produced water contains a toxic mixture of benzene, arsenic, lead, toluene, and varying amounts of radioactive pollutants (Fakhru'l-Razi *et al*., 2009). It is estimated that hundreds of thousands of gallons of this produced water are discharged daily from each oil platform, leading to the pollution of both local waters and those down current from the discharge (Clark and Veil, 2009).

1.2.5 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are a unique class of ubiquitous compounds that consist of fused conjugated aromatic rings and constitute 0.2% to 7% crude oil (German Federal Environment Agency, 2012). They are ubiquitous in the sense that they can be found in appreciable amounts in such remote locations as Arctic ice and snow, high altitude lake sediments and deep-sea sediments (Lima *et al*., 2005). "The sources and environmental fate of PAHs has been the subject of extensive studies due to the carcinogenic and/or mutagenic

properties of several of their isomers (e.g., benzo[*a*]pyrene). Therefore, the concentration and sources of these compounds are closely monitored" (Lima *et al*., 2005)**.**

PAHs are usually constituted from four-, five-, six- or seven-member aromatic rings, with the five or six ringed members being the most widespread (Pietzsch *et al.*, 2010). PAHs formed from only six-membered rings are known as alternants with some alternants being classified as benzenoid, a name derived from benzene(an aromatic hydrocarbon with a single, six-membered ring) (Lundstedt *et al*., 2007). Equally distributed electron density characterizes alternant PAHs. PAHs are classified as "small" when they contain two to three fused aromatic rings and "large" when they are constituted by more than three aromatic rings (Nyarko *et al.*, 2011). There are about 100 PAHs that have been identified but only 16 of these attract public interest (Hussar *et al.*, 2012). Chemical structure of 20 PAHs are shown in Figure 1.2 and Figure 1.3 shows 2 examples of carcinogenic PAHs (the arrows indicate bay and fjord regions, a property of carcinogenici PAHs).

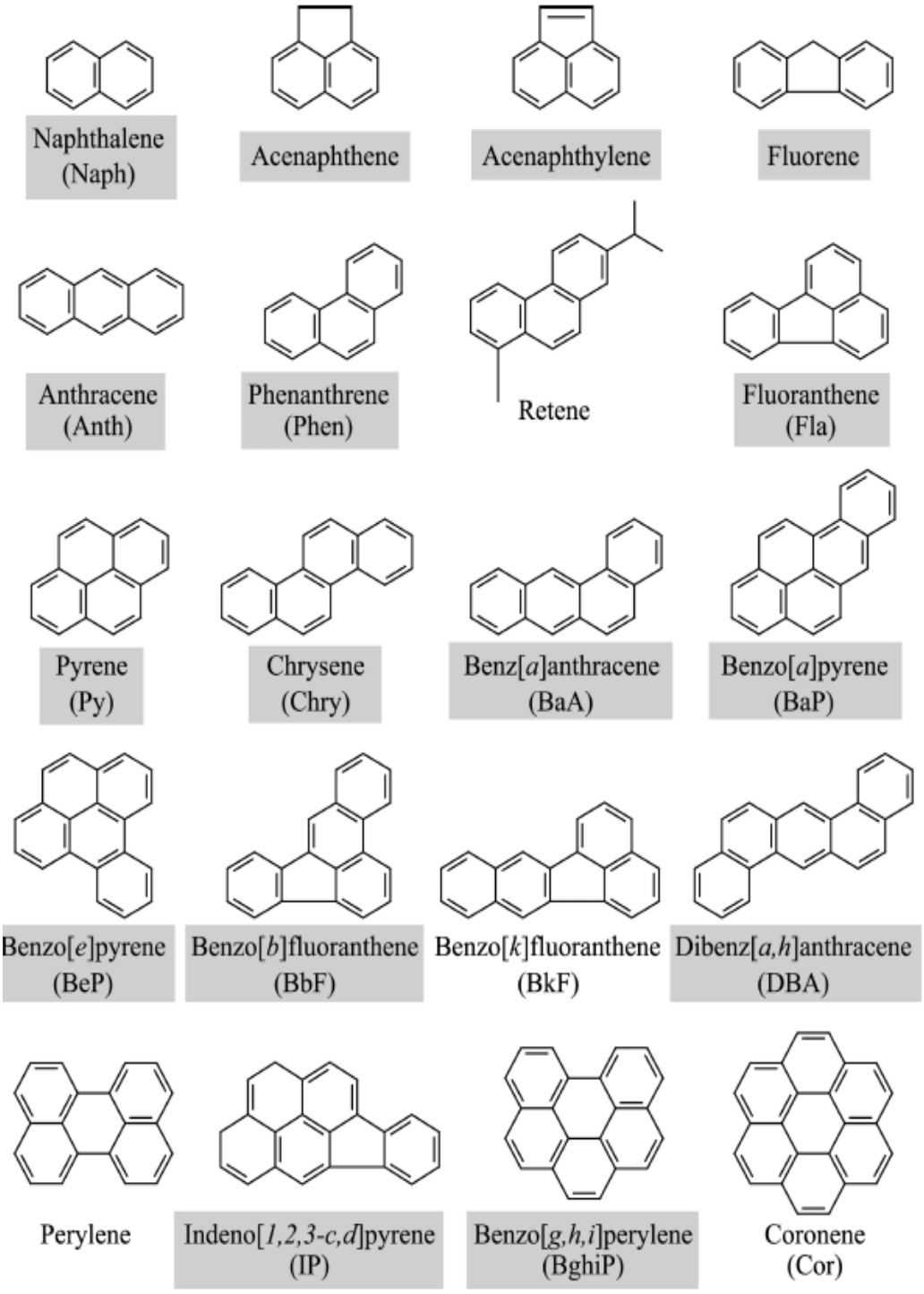

Figure 1. 2 Chemical structure of some PAHs. Those highlighted among the ones classified as toxic. (Lima *et al.*, 2005)

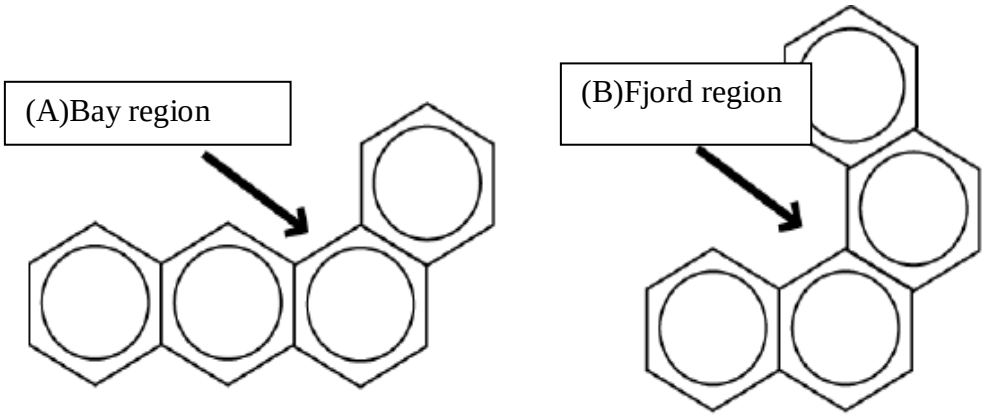

Figure 1. 3 Typical examples of carcinogenic PAHs such as benz[a]anthracene and benzo[c]phenanthrene with the arrows. (Muñoz and Albores, 2010)

1.2.6 Sources of PAH Pollution

The release of PAHs into the marine and coastal environment occurs via natural and anthropogenic sources. Natural sources include oil seeps, volcanoes, grass fires, activities of chlorophyllous and nonchlorophyllous bacteria and fungi (Sakari, 2004). Anthropogenic sources of PAH pollution of the coastal and marine environment can largely be attributed to urban runoff, wastewater effluents, industrial outfalls, atmospheric deposition, spills and leaks during the transport and production of fossil fuels (Latimer and Zheng, 2003). The concentration of PAHs have been observed to be very high in estuaries and coastal environments closer to urban areas and this is particularly due to the influx from sources localized in coastal environment (Simcik *et al*., 1999). Usually PAHs from these sources are found deposited in soil, sediment and oily substances and to a comparatively lower extent in water or air (Patrolecco *et al*., 2010). Of all the anthropogenic sources, those from petroleum and its products (petrogenic sources) constitute the largest source of PAH pollution in coastal

environments especially where offshore drilling activities are taking place (Wang *et al*., 2007). Petroleum and its products which originate from concentrated hydrocarbon sources enter the marine environment and subject to dispersion, evaporations, settlement in the bottom on the sediments, weathering, chemical changes, sunlight effects (photooxidation) and microbial degradation (bacteria, yeast and fungi) in short and long term period (Sakari, 2004).

Incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco, and incense also contribute a significant amount of PAHs that pollute the environment (Saha *et al*., 2009). The combustion derived PAHs are collectively classified as pyrogenic PAHs. Several important differences exist between PAHs from combustion (pyrogenic) sources and PAHs from petroleum (petrogenic). In a very general sense, combustion sources tend to be relatively enriched with higher molecular weight PAHs (e.g., four- to six-ring PAHs) whereas petrogenic sources tend to be relatively enriched with two and three-ring PAHs (Burgess *et al*., 2003). However, a more distinguishing difference between pyrogenic and petrogenic sources can be seen in the abundance of parent PAH compounds relative to their alkylated homologs within a given homolog series. As a general rule, alkylated PAHs are less abundant than the parent compound in environmental samples influenced by pyrogenic sources, whereas alkylated PAHs are typically more abundant than the parent compound in environmental samples influenced by petrogenic source (Burgess *et al*., 2003).

1.2.7 Physicochemical Properties of PAHs

Physicochemical properties of PAHs such as resistance to redox reactions, melting point, boiling point, aqueous solubility and volatility vary with molecular weight and ultimately influence their deposition and distribution in the coastal environment, and their effects on biological systems (Bihari *et al.*, 2006). Aqueous solubility of PAH decreases with increasing molecular weight. Thus, for unsubstituted PAHs, solubility range from slightly soluble naphthalene to highly insoluble benzo[ghi]perylene (Zemanek *et al*., 1997). The solubility of PAHs is also affected by the alkyl (e.g. CH_2- group) substitution on the aromatic ring. Generally there is an overall decrease in aqueous solubility of PAHs with respect to an alkyl group substitution as compared to its unsubstituted form but, benz[a]anthracene is an exception to this rule as it is less soluble than either methyl- or ethylbenz[a]anthracene (Mei *et al*., 2009). PAHs with linear arrangements are less soluble in water than angular or perifused molecules as is exemplified by anthracene being less soluble than phenanthrene (Mei *et al*., 2009). Naphthalene is also less soluble in water than chrysene or benz[a]anthracene due to linear form of naphthalene (Burgess *et al*., 2003).

Vapor pressure or volatility is another important characteristic of PAHs due to the fact that it determines the persistence of PAHs in the aquatic environment (Wang *et al*., 2001). The vapor pressure of PAHs increases as the number of rings decreases. Hence 2 or 3 ring PAHs are very volatile, while PAHs with 4 or more rings show insignificant volatilizational loss under all environmental conditions. Naphthalene also has the highest vapour pressure relative to the non-volatile dibenzo[ah]anthracene (Ravindra *et al*.,

2006). PAHs such as naphthalene which are light molecular weight PAHs and volatile easily pass through soils and contaminate groundwater supplies (ATSDR, 1995) but other high molecular weight non-volatile PAHs such as benzo(b)fluoranthene adhere very strongly to soil and organic matter.

Due to their hydrophobic nature, PAHs entering the aquatic environment exhibit a high affinity for suspended particulates in the water column (Cofield *et al*., 2007). As PAHs tend to adsorb to these particles, they eventually settle out of the water column onto the bottom sediments. Thus, the PAH concentrations in water are usually quite low relative to the concentrations in the bottom sediments (Cofield *et al*., 2007). PAHs also exhibit characteristic UV absorbance spectra with many absorbance bands that are unique for each ring structure (Malloci *et al*., 2004). This is particularly useful in the identification of PAHs during HPLC analysis. They are also able to fluoresce with characteristic wavelengths of light when excited (when the molecules absorb light). These characteristic absorbance spectra are due to an extended pi-electron electronic structure of PAHs (Ruiterkamp *et al*., 2002).

1.2.8 Human Exposure to PAH

Routes of human exposure to PAHs include ingestion, inhalation, and dermal contact in occupational and non-occupational settings (Ravindra *et al*., 2006). Thus, human exposure to PAHs can largely be attributed to breathing polluted air, eating food containing PAHs, smoking cigarettes, or breathing smoke from open fireplaces (ACGIH, 2005). Various means of processing food such as smoking and cooking of foods at high temperatures (grilling, roasting, and frying) constitute major sources of generating PAH

thus leading to the ingestion of this toxicant (Chen and Lin, 2001). Plants are also able to take up PAH from the soil through their roots. Surface water bodies are usually contaminated by PAH through deposition but underground water is usually contaminated through leaching of PAHs from those deposited in soil.

Another means by which humans can be exposed to PAHs could be via contact with contaminated soil through ingestion, inhalation, or dermal (skin) exposure (Wang *et al*., 2012). Workers such as those involved in mining, metal working, or oil refining are at risk of inhaling air contaminated with PAHs due to long working hours (Armstrong *et al*., 2004; See *et al*., 2006).

1.2.9 Health Effects of PAH

The effects of PAH on the health of humans can result from short term (acute) exposure to PAH or long term (chronic) exposure.

1.2.9.1 Acute or Short-Term Health Effects

Acute exposure to PAHs has been reported to induce impaired lung function in asthmatics and thrombotic effects in people with coronary heart disease (ACGIH, 2005). The acute effects of PAHs on human health are deemed to be dependent mainly on the extent of exposure, the concentration of PAHs during exposure, the toxicity of the PAHs, and the route of exposure, e.g., via inhalation, ingestion, or skin contact (ACGIH, 2005). PAHs have been reported to cause skin irritation and inflammation (Unwin *et al*., 2006). PAHs such as anthracene, benzo(a)pyrene, and naphthalene are known to be skin irritants, while anthracene and benzo(a)pyrene are reported to be skin sensitizers, i.e. cause allergic skin response in animals and humans (IPCS, 2010).

1.2.9.2 Chronic or long-term health effects

Long term exposures to PAHs have been reported to cause health conditions with different level of severity. Occupational exposure to PAHs and other work place chemicals, result in a series of health problems such as an increased risk of skin, lung, bladder, and gastrointestinal cancers (Talaska *et al*., 1996). Reproductive and developmental effects from PAH exposure have been documented in humans and incidence of such health conditions has also been reported in animal studies (Wells *et al*., 2010).

1.2.9.2.1 *Carcinogenicity and Mutagenicity*

The connection between PAH and the occurrences of cancer begun in 1775 with Percival Pott when he noticed a high incidence of scrotal cancer in chimney sweeps (Hall, 1998). He first postulated that the cause was chemicals contained in the soot on which studies conducted by Yamagiwa and Ichikawa in 1918 using rabbits painted with coal on their ear, for their experiment confirmed the development of skin cancer (Fu *et al*., 2012) . Various soots, tars, and oils were subsequently shown to be carcinogenic in humans and laboratory animals, and such complex mixtures were later found to contain rich sources of polycyclic aromatic hydrocarbons (PAHs), including the potent carcinogen, benzo[a]pyrene (Fu *et al*., 2012).

Based on these findings other epidemiological studies undertaken also established a strong association between PAH exposure and breast cancer (Rundle *et al*., 2000). From one of the studies (conducted as part of the Long Island Breast Cancer Study Project), 50 percent greater risk of breast cancer in women was linked to the highest level of PAH-DNA adducts (Gammon *et al*., 2002). PAH-DNA adducts are indicators of problems in

DNA repair in cells; one of the early hallmarks of tumour development. The presence of PAH-DNA adducts in breast tissue samples taken from women diagnosed with cancer were found to be as much as twice the levels in those diagnosed with benign breast disease (Rundle, 2000). The carcinogenic activities of PAHs has been found to be largely dependent on their structural characteristics such as the number of rings, the sites of fusion, extent of condensation, and degree and site of methylation (Ross and Nesnow, 1999).

PAHs are not carcinogenic themselves, but are only able to induce cancer upon metabolic activation by the mixed function oxidase–cytochrome P-450s present in high concentration in liver tissues, and to a lesser extent in other tissues (Xue and Warshawsky, 2005). These PAHs are converted to their reactive forms that covalently bind to DNA and form DNA adducts (Xue and Warshawsky, 2005). These reactive arene oxides are usually hydrolyzed by microsomal epoxide hydrolase to a trans-dihydrodiol and then subsequently conjugated by phase II enzymes to be excreted (Schwarz *et al*., 2001). But when the oxygen insertion occur on carbons within the bay-region or fjord-region of the PAH, the epoxide hydrolase is unable to hydrolyze these arene epoxides due to steric hindrance from the adjacent dihydrodiol group (Parkinson and Ogilvie, 2008). A feature common to all bay-region epoxides is their resistance to hydrolyation by epoxide hydrolase, which results from steric hindrance from the nearby dihydrodiol group. Extremely reactive arene diolepoxides can be generated which then bind strongly to DNA to form adducts or induce oxidative stress that provokes mutations (Parkinson and Ogilvie, 2008). Figure 1.4 is a schematic representation of the 3 pathways of

bioactivation of BaP. The point of interest in this pathway is the epoxidation of BaP, subsequent hydroxylation by epoxide hydrolase and further epoxidation by CYP or PHS to its DNA binding form.

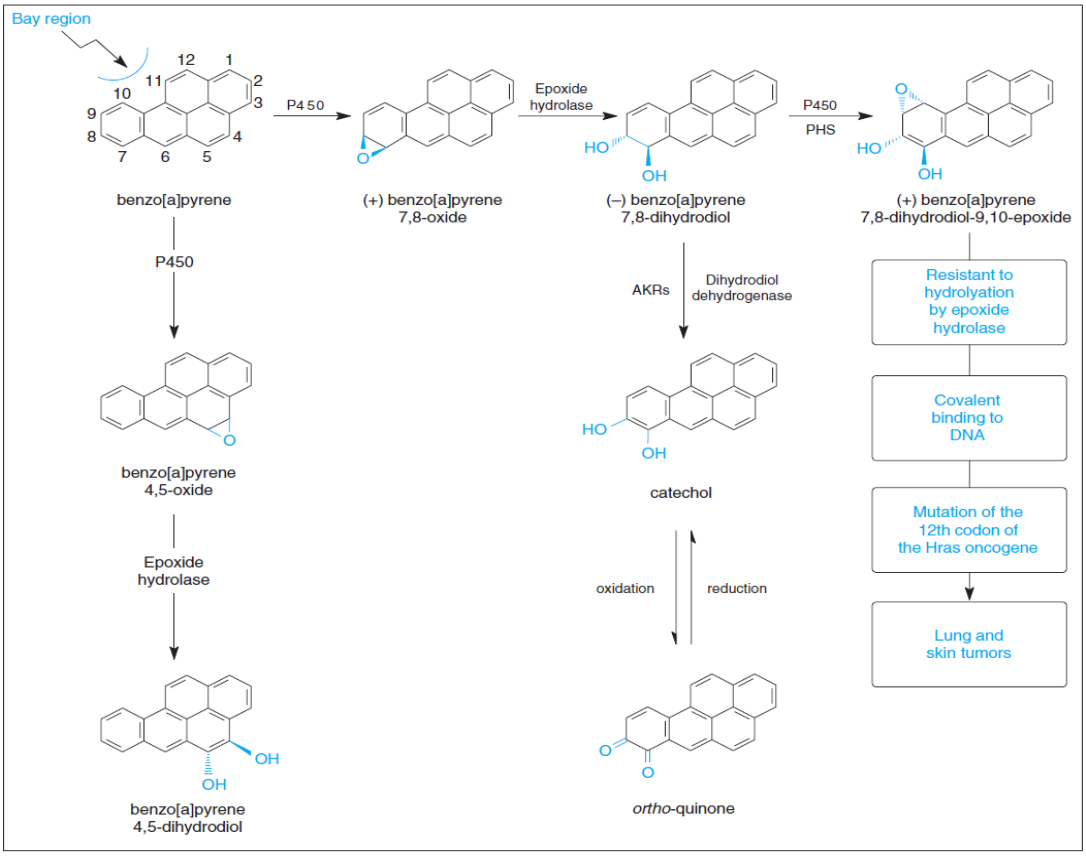

Figure 1. 4 A schematic representation of three pathways of bioactivating BaP to its DNA binding form benzo[a]pyrene 7,8-dihydrodiol-9,10-oxide. (Parkinson and Ogilvie, 2008).

The mutations are provoked when DNA repair mechanisms are damaged by the rate of adduct formation and excessive generation of ROS species, as opposed to scavenging of these molecules (Roy *et al.*, 2012). These conditions which afflict DNA repair mechanisms ultimately lead to accumulation of mutations in DNA that may induce carcinogenesis (Ziech *et al.*, 2011). The bulky nature of adducts in DNA obstruct the

replication activity of polymerase enzyme thereby causing reduction in repair activity thus contributing to increased DNA damage (Federley and Romano, 2010). These mutations result in carcinogenesis when they occur in genes whose product regulates cell survival (Hang, 2010). A typical example of genes in question is the p53 protein gene which is a transcription factor that regulates cell proliferation, differentiation, apoptosis, and DNA repair (Muller and Vousden, 2013). Another common target of mutagenesis induced by PAH-DNA adduct is the ras gene (Ross and Nesnow, 1998). A study by Gray *et al*. (2001) revealed that exposure of mice to BaP lead to increased in mutation of the K-ras gene.

PAHs are able to induce an increased transcription of CYP1A1 and CYP1B1 by binding to a protein (Figure 1.5) called the aryl hydrocarbon receptor (AhR) (Ma and Lu, 2007). This cytoplasmic AhR is usually found in complex with other proteins such as heat shock protein 90 (Hsp90), p23 and AhR-interacting protein (Heid *et al*., 2000). After the binding of PAHs to AhR, the Hsp90 is released and the AhR-PAH complex translocates to the nucleus where it heterodimerizes with a Ah Receptor Nuclear Translocator (ARNT) (Sogawa *et al*., 1995). This AhR-ARNT complex binds to DNA via the xenobiotic response element (XRE) situated in the promoter region of CYP1A and CYP1B genes and induce their rapid transcription (Kinoshita *et al*., 2004). Increased concentrations of CYP1A and CYP1B are then able to bioactivate these bay and fjord-region containing PAHs to generate their highly reactive DNA binding forms, thus leading to increased adduct formation and ROS generation, ultimately leading to mutations and cancer (Amin *et al*., 2003).

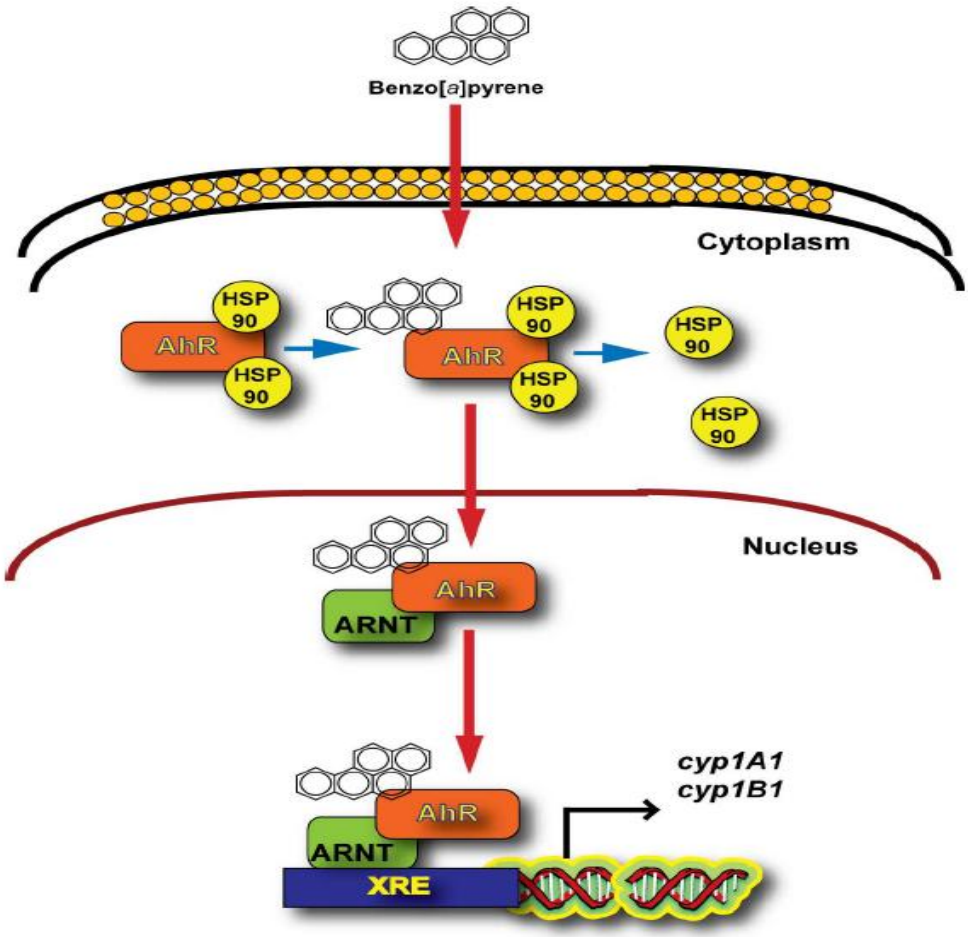

Figure 1. 5 Pathway depicting the induction of CYP1A1 and CYP1B1 gene transcription by PAHs via the AhR (Muñoz and Albores, 2010)

1.2.9.2.2 *Teratogenicity*

A number of studies conducted provide conclusive evidence of PAH being teratogenic in laboratory animals. Wassenberg and Di Giulio (2004) report PAHs such as benzo(a)anthracene, benzo(a)pyrene, and naphthalene as being embryotoxic in mice. Birth defects such as decreased body weight was observed in mice offsprings when these mice were fed with benzo(a)pyrene during pregnancy (Kristensen *et al*., 1995).

Another study conducted by the US Center for Children's Environmental Health (CCEH) reported that exposure to PAH pollution during pregnancy is related to adverse birth

outcomes, including low birth weight, premature delivery, and delayed child development (Perera *et al*., 2005). Defects such as low intelligent quotient (IQ) at age three, increased behavioral problems at ages six to eight, while childhood asthma has been associated with high prenatal exposure to PAHs (Perera *et al*., 2006).

Congenital defect known as gastroschisis which is characterized by a defect in the anterior abdominal wall through which the abdominal contents freely protrude has been reported to occur in babies due to exposure of their mothers in age bracket of 20 years and above to PAHs (Lupo *et al*., 2012). Yuan *et al*. (2013) reported the association between the low levels of placental PAH-DNA adducts with an increased risk of neural tube defects.

1.2.10 Biomarkers of PAH Pollution

The coastal environment is usually modified by a variety of organic and inorganic pollutants from natural or anthropogenic sources. These pollutants such as PAH have the potential of inducing damages to the aquatic biota at molecular, biochemical, cellular and physiological levels (Valavanidis and Vlachogianni, 2010). The continuous input of pollutants into the environment has spurred the development of different techniques to evaluate and monitor their fate and effects (Seriani *et al*., 2012). Fishes are normally used as model sentinel organisms for biological monitoring of the environment because they provide a sensitive and reliable approach to estimate the potential effects of pollutants providing early warning for pollution-induced environmental changes (Sarkar *et al*., 2006). Fish have similar manner of bioactivating xenobiotics as other vertebrates, and

this may be exploited to evaluate the potential of some contaminants to produce teratogenic and carcinogenic effects in humans (Al-Sabti and Metcalfe, 1990).

1.2.10.1 Micronucleus Test

Micronucleus assay is the most widely applied method to detect genotoxicity and cytotoxicity since it is easy to carry out, quick and reliable (Galindo and Moreira, 2009). Micronuclei as genotoxicity or cytotoxicity indicators are basically cytoplasmic chromatin masses which have the appearance of small nuclei that crop up from chromosome fragments or intact whole chromosomes that lagged behind in the anaphase stage of cell division (Luzhna *et al*., 2013).

Any active tissue can be employed for this assay because micronuclei are easily observed in both somatic and germinal cells (Udroiu, 2006). However, the use of hepatic cells for micronucleus test is considered appropriate for chemicals such as PAH that require metabolic activation to become genotoxic (Fenech, 2000). The application of this assay to erythrocytes is very popular, in particular with blood samples, as thousands of scorable cells are present in blood (Udroiu, 2006). Therefore, the application of the micronucleus test on peripheral blood samples is particularly a good indicator for conditions of chronic exposure as erythrocytes from the peripheral circulation reflect events that occurred in a time equal to the lifespan of the circulating erythrocytes (Udroiu, 2006). A meaningful inference can be made from this assay by comparing the mean adduct observed per thousand erythrocytes in fish from ‘control’ and ‘exposed’ sites (Udroiu, 2006).

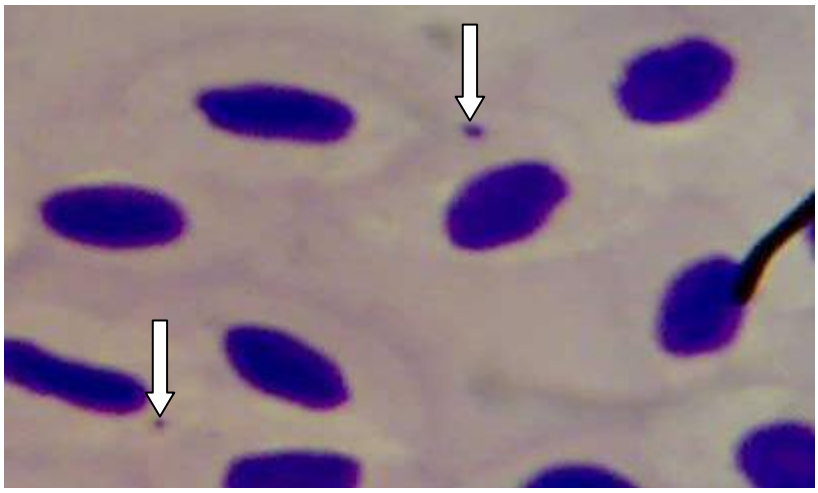

Figure 1. 6 Micronucleated cells in erythrocytes (Arrowed)

(Ahmad and Saleh, 2010).

1.2.11 Method of PAH Extraction

Solvent extraction of PAH can be accomplished althrough a wide variety of techniques commonly used for extracting hydrocarbons from soils, plants, water sediments and animal tissues (Lau *et al*., 2010). Conventional extraction techniques such as Soxhlet, ultrasonication, mechanical and reflux with methanolic KOH are frequently employed for PAH extraction (Lau *et al*., 2010). But in recent times a number of automated techniques are frequently used for extractions; some of which include soxtec (automated soxhlet), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) pressurized hot water extraction (PHWE) pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) (Lau *et al*., 2010). The choice of a particular method to use for efficient extraction can be made based on techniques that can produce good results within a short time with minimum operator involvement. The cost involved should be minimal and most importantly the technique should be safe for both the analyst and environment (Guerin, 1999).

The efficiency of these extraction methods are ascertained by comparison with Soxhlet extraction which is the standard and the preferred method for extracting semi-volatile and non-volatile organics from solid matrices (Guerin, 1999). Environmental monitoring agencies usually prefer this method as it is an easily standardized technique with high recoveries, compared to matrix dependent techniques such as MAE, PLE and SFE (Shen and Shao, 2005). A major setback with soxhlet extraction is the duration of the procedure (20 h or more) and the use of large volumes of organic solvents (Luque de Castro and Priego-Capote, 2010). In addition, it is a tedious process, which more often than not leads to the thermal degradation of labile compounds. There is the need for efficient, quick, cheap and less solvent requiring method for routine environmental monitoring of pollutants such as PAH.

Extraction by ultrasonication method is an efficient technique when compared to Soxhlet extraction method for extracting trace organics from environmental samples such as plants, soils and sediments (Song *et al*., 2002). This method uses the acoustic energy of ultrasonic waves with a minimum frequency of 16 kHz in fluid, to cause rapid compression and rarefaction of fluid movement resulting in the cavitation phenomenon, that is, the re-occurring formation and collapse of microbubbles (Song *et al*., 2002). The agitation is achieved either by immersing a sonicator transducer also known as an ultrasonic horn into the sample solvent mixture or placing the sample solvent mixture directly into a sonication bath. The desired ultrasound is generated by means of

piezoelectric ceramic attached either to the ultrasonic horn or the walls of the sonication bath (Wu *et al*., 2001).

A more cost-effective sample preparation method is the solid phase extraction (SPE) which is a faster and time saving over many traditional liquid/liquid extraction techniques (Biziuk, 2006). The principle behind this method is basically the retention of the analytes to be determined and/or the interferences of the samples on the sorbent by different mechanism (Lau *et al*., 2010). Elution of the analytes in the sample are best achieved in a small volume of a solvent which also aids in cleaning up and concentrating the analytes (Marcé and Borrull, 2000).

1.2.12 Regulation of PAH Levels in the Environment

Due to the toxic nature of PAH, a number of governmental agencies with oversight responsibilities for monitoring the environment have established standards that are relevant to PAH exposures in the workplace and the environment (Harper, 2004). The recommended exposure limit (REL) of 0.1 mg m^{-3} has been set by the US National Institute for Occupational Safety and Health (NIOSH) for a 10-hour workday or 40-hour workweek at the workplace (NIOSH, 2010). Also maximum contaminant level (MCL) of 0.2 ppb is set for benzo(a)pyrene, the most carcinogenic PAH with respect to its levels in water (US EPA, 2000a). The unit risk of lung cancer of BaP as reported by the World Health Organization (WHO) is 87×10^{-6} ng m^{-3} for lifetime exposure but some member states of WHO have set the guideline values for BaP between 0.1 and 1.3 ng m^{-3} (WHO, 2003).

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and Reagents

PAH Mix 14 Standard was obtained from Dr. Ehrenstorfer GMBH Augsburg, Germany. Silica Gel was from Merck, Germany. Acetone was purchased from Prolabo VWR BDH, Singapore. Dichloromethane (DCM), Ethanol, Hexane, Methanol, DPX Mountant, Maygrunnwald, Acetonitrile, and Giemsa Stain were obtained from Sigma Aldrich St Louis,USA. Sodium Sulfate Anhydrous [Na_2SO_4], Copper (II)Sulfate Pentahydrate [$CuSO_4.5H_20$], Phosphoric Acid [H_3PO_4], were obtained from Wako Pure Chemicals Industries Limited Japan.

2.2 METHODS

2.2.1 Study Area

This baseline study on environmental impact assessment of crude oil drilling was conducted in six districts bordering the marine environment of the Jubilee Oil field in the Western Region of Ghana. The Western Region covers an area of 23,921 km^2, which forms about 10% of Ghana's total land area (Western - Government of Ghana, 2015). It is located in the south-western part of Ghana. It 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 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 Republic of Cote D'Ivoire borders to the estuary of Ankobra.

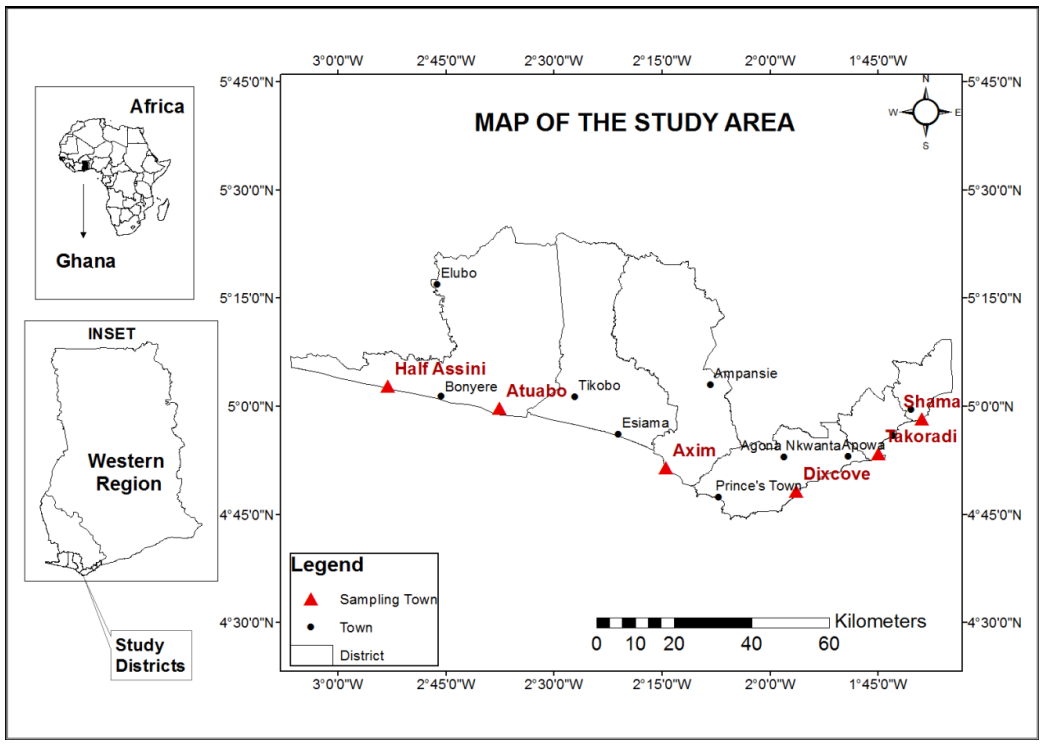

Figure 2. 1 A map of the study areas in the Western coast of Ghana.

The 2010 population and housing census, estimated the total population of the region 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 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%.

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.

2.2.1.1 Ellembelle District

It is located in the southern end of the region between longitudes 2º05' W and 2º35' W and latitude 4º40N and 5º20N. It covers a total land area of 1468 km^2. It shares boundaries with Jomoro District to the west, Wassa Amenfi West District to the north, Nzema East Municipal to Tarkwa – Nsuaem Municipal to the East and 70 km stretch of sandy beach to the south. The district lies within the wet semi-equatorial climate zone. The population of the district is 87,501 according to the 2010 Population and Housing Census (Ghana Statistical Service report, 2012). The area experiences rainfall throughout the year with the highest monthly mean rainfall occurring around May and June. The mean annual rainfall is 1,600 mm and the average temperature is about 29°C. The vegetation of the area is made up of 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. Fishing is the major industry in this district.

2.2.1.2 Jomoro District

The district is located between latitudes 040 55' N and 050 15' N and longitudes 020 15' W and 020 45' W. It covers a land area of 1344 km^2 and shares boundaries with Wassa Amenfi and Aowin-Suaman 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 2010 population census is 150,107 (Ghana Statistical Service report, 2012). The rainfall is double maxima occurring from April to July and September to November. There is also a short dry spell in August and a longer dry period in December to January. The average annual rainfall is 1732 mm. The climate of the area is classified as equatorial monsoon. The vegetation is tropical rainforest with coastal vegetation being largely mangrove swamp ("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 majority of the population who obtain their livelihood from farming and other agro related activities such as deep sea and fresh water fishing as well as coconut oil extraction.

2.2.1.3 Nzema East Municipality

It covers a land area of 2194 km^2. It 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. 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 rain forest 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.

2.2.1.4 Ahanta West District

The district lies between latitude 4°.45"N and longitude 1°.58"W. It covers a total land area of 591 km^2 and it is occupied by 106,215 people 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 the north by Mpohor Wassa East and Tarkwa Nsuem Districts and the Gulf of Guinea to the south. The climate of the area 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 rain forest vegetation zone. The topography is generally low-lying.

2.2.1.5 Shama District

It covers a land area of 215 km^2 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 to 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 raining season starts from March to mid-July followed by short dry spell that run till the end of 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 22 ℃ to 28 ℃. 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 the 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 made up of 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.

2.2.1.6 Sekondi-Takoradi Metropolitan District

It is located in the south-western part of Ghana and it covers a land area of about 49.78 km^2. It is bordered to the east by Shama District, to the north by Mpohor Wassa East

District, to the west by Ahanta West District and to the south by Gulf of Guinea. The population of the metropolis 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 22℃. 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 geology of the area is underlain with faulty shale and sandstone on granite, gneiss and schist (Western - Government of Ghana, 2015).

2.2.2 Experimental Design

In order obtain baseline data on environmental impact based on proximity to the Oil fields, communities were clustered into three according to distance from the Jubilee field: under five kilometers (Dixcove in Ahanta West District and Lower Axim in Nzema East Municipality), 5-10 kilometers (Atuabo in Ellembelle District and Half Assini in Jomoro District) and over ten kilometers (Aboadze in Shama District and New Takoradi in Sekondi-Takoradi Metropolis) onshore of the Oil fields. Samples were collected from a randomly selected community in each district. In community five sampling sites were randomly selected 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.

2.2.3 Inclusion and Exclusion Criteria

Coastal communities bordering the Jubilee oil field in the Western region of Ghana were included in the study. Water samples were not collected from water bodies which did not serve any domestic purpose in each particular study area. Leaves were taken from plant species which were dominant in each community. Only fresh fish were acquired from the various landing beaches in each study area.

2.2.4 Sample Collection

In this study, soil, water, plant and fish samples were collected from the six study sites.

2.2.4.1 Soil Samples

Soil samples were taken from 5 different locations from the shores and in-land of selected susceptible community at depths of 0 to 15 cm and 15 to 30 cm (a total of ten soil samples for each of the six study areas). The samples were subsequently stored in plastic containers for PAH extraction and analysis.

2.2.4.2 Water Samples

Sample bottles for the assessment of PAH were cleaned and rinsed using distilled water only. Water was collected from 5 different locations, transferred into the bottles and stored at 4°C until PAH analysis was done. No water samples were obtained from Shama and Sekondi-Takoradi Metropolitan Districts. This is because water bodies in these places 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.

2.2.4.3 Plant and Fish Samples

Plant samples were taken randomly from areas in selected communities close to the shore and in-land. They were kept in zip lock plastic bags and stored at 4^{o}C until use, for PAH analysis. The plant samples selected were dominant plants in a particular sampling cluster. The different species of plants sampled for this study are as listed in Appendix A3 and A4. 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 sampled for this project.

The number of fish species sampled differed from district to district as shown in table A1 and A2 but in all 38 fish samples (three per each species making a total of 114) was collected from the entire selected cluster or fishing community for PAH extraction and micronucleus test. 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. The samples were transported on ice to the laboratory and stored at -20^{o}C until use.

2.2.5 Determination of PAHs Concentration

Polycyclic aromatic hydrocarbons (PAHs) concentration in plants, soil and water samples were analyzed according to the method prescribed by Li *et al*. (2009). PAH content in fish were analyzed as described by Denton *et al*. (1999) with modifications.

2.2.5.1 Preparation of Samples

2.2.5.1.1 Plants

Each fresh plant sample was ground into a homogenous mixture with the aid of a pestle and mortar. Two grams of homogenized leaf samples were placed in a 100 ml conical glass flask containing 5 ml acetone, 0.1 g copper powder and 5 g of anhydrous sodium sulphate. Extraction was carried out for 15 min in an ultrasonic water bath at a temperature of 30°C. The mixtures were placed in 15 ml centrifuge tubes and centrifuged at 2190 rpm for 10 min and the supernatant, were decanted into fresh 15 ml centrifuge tubes. The extraction process was repeated by adding 5 ml of acetone to the residues and the content transferred into glass flasks before sonicating. The supernatants were then pooled together and evaporated to dryness in a rotary evaporator. Concentrated plant extracts from the rotary evaporation process were then subjected to further extraction by column chromatography.

2.2.5.1.2 Soil

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

2.2.5.1.3 Fish

Fish livers of the same species were removed from storage and thawed prior to extraction. Using stainless steel scissors and forceps, 1.5 g each of tissue samples was

weighed into a 50 ml centrifuge tube. Subsequently, 10 g anhydrous, granular sodium sulfate, and 10 ml of dichloromethane were added to each tissue sample before homogenization using a mortar and pestle. The mixture was placed in a 50 ml centrifuge tube and centrifuged at 3500 rpm for 20 min before decanting the supernatant 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 by means of rotary evaporation, the extract was transferred to a 15 ml centrifuge tube with two 0.5 ml rinses of dichloromethane. The tube was placed in a water bath and the extract volume reduced to 0.25 ml under a gentle stream of nitrogen. Each extract was then dissolved in 2 ml of hexane and further reduction in volume to 1ml was done before further extraction by column chromatography.

2.2.5.1.4 Water

A solid phase extraction (SPE) cartridge system was used to extract PAHs from the water samples. Prior to extraction, the C18 cartridges were pre-conditioned with 5 ml acetonitrile, followed by 10 sec vacuum drying and washed with 5 ml of HPLC grade ultra pure water. 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.

2.2.5.2 Column Chromatographic Extraction of PAHs

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

2.2.5.2.1 Plant, soil and water samples

Ten glass columns of 1.5 mm internal diameter 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 then securely clamped and supported on a stand after which the taps were closed. This was followed by filling the columns to one third of their volume with dichloromethane and hexane (1:1 v/v) as a mobile phase. Slurry of silica 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 the aid of a Pasteur pipette and the solvent was allowed to drain to prevent overflowing. The columns were gently tapped with a rubber bung to free it of air bubbles. This was followed by draining the solvent until its level was just even with the surface of the stationary phase after which the tap was closed. The concentrated samples from the rotary evaporation were then dissolved in 2 ml of mobile phase. Subsequently, 1 ml of each sample was loaded into a separate packed column. Each sample was then eluted with 8 ml of mobile phase and the eluents were collected in labelled test tubes. This was followed by evaporating the eluted samples to dryness under a gentle flow of nitrogen gas. 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.

2.2.5.3 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. 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 was made up of water and phosphoric acid (component A) and acetonitrile (component B). Details of the mobile phase are given in Table 2.1. Analyte (PAHs) peaks were identified by their retention times compared to the corresponding retention times of the PAH standards used.

Table 2.1: 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

2.2.6 Genotoxicity Assessment

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

2.2.6.1 Micronucleus Assay

Peripheral blood samples were obtained from the caudal vein of the fish specimen. Thin smears were made on pre-cleaned slides. The slides were then air-dried at room temperature for 24 h in a dust and moisture free environment in paper boxes. Smears were fixed by dipping slides into methanol for 5-10 min. Following air-drying of slides for 1 h, smears were stained with Maygrunwalds stain solution-I for 2-3 min, washed with double distilled water and dried. Immediately after drying, smears were stained with Maygrunwalds stain solution-II for 3-6 min, washed with double distilled water and dried. 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 permanently fixed with DPX mountant and dried overnight. Cells (normal and abnormal) and micronucleus were then observed and counted under a microscope (Leica, Germany) at magnification 100.

The mean frequency of micronuclei per 1000 cells of each fish species was determined based on the following scoring criteria; (1) the micronucleus had the same staining characteristics and similar morphology to the main nuclei, (2) any micronuclei present was separate in the cytoplasm or only just touching a main nucleus, (3) micronuclei were smooth edged and smaller than approximately one third the diameter of the main nuclei.

The frequencies of micronuclei were computed for each species using the equation below.

$$\textbf{MNE } (^{0}/_{00}) = \frac{\text{Number of cells containing micronuclei}}{\text{Total number of cells counted}} \times 1000 \quad \textbf{.....Equation 2.1}$$

2.2.7 Statistical Analysis

Results were expressed as means ± standard deviation (SD). Concentrations of soil and plants were expressed as mg/kg wet weight whiles concentrations of fish were expressed as µg/g and that of water expressed as µg/L. The results of 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).

CHAPTER THREE

3.0 RESULTS

3.1 CONCENTRATION OF PAHS

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

3.1.1 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 computed from the data obtained from duplicate HPLC analysis of composite samples each fish species (three fish per species).The liver of three fishes for each species were pooled together for extraction prior to HPLC analysis.

Nine different fish species(three per each species) namely: *Illisha africana, Sardinella eba, Sardinella aurita, Brachydeuterus auritus, Pomadasys incises, Dentex congoensis, Pseudupe prayensis, Lutjanus fulgens and Katsuwonus pelamis* were sampled from Aboadze landing beach in the Shama District. Only *P. incises* had naphthalene (6.933 µg/g), acenaphthylene (15.267 µg/g), 2-methylnaphthalene (5.667 µg/g) and acenaphthene (0.333 µg/g) detected as shown in figure 3.1. No PAHs were detected in the other fish species from the community.

With respect to Dixcove in the Ahanta West District, the fish species *Trichiurus lepturus, Thunnus albacores, Katsuwonus pelamis and Thunnus alalunga* were sampled. However, only fluorene with a concentration of 0.180 µg/g was detected in *T. alalunga* fish as shown in figure 3.1.

Fish species: *Galeoides decadactylus, Pentanemus quinquarius, Pseudotholitus senegalensis, Brachydeuterus auritus* and *Illisha Africana* from New-Takoradi landing beach in the Sekondi-Takoradi Metropolitan District showed no detectable PAHs. In Lower Axim in Nzema East District no PAHs were detected in the fish species: *Brachydeuterus auritus, Dentex angolensis, Chloroscombrus chrysurus, Sphyraena sphyraena, Stromateus fiotola and Pseudotolithus typus* sampled.

Also no PAHs was found in *Caranx chrysurus, Seriola dumurili, Ethmalosa dorsalis, Sphyraena sphyraena, Selene dorsalis* and *Caranx hippos* fish species sampled from Atuabo in the Ellembelle District. Likewise, no PAHs were detected in fishes: *Brachydeuterus auritus, Sphyraena sphyraena, Sardinella aurieta, Hemiramphus brasiliensis, Pomadasys incises and Acanthurus monroviae* from Half Assini in the Jomoro District.

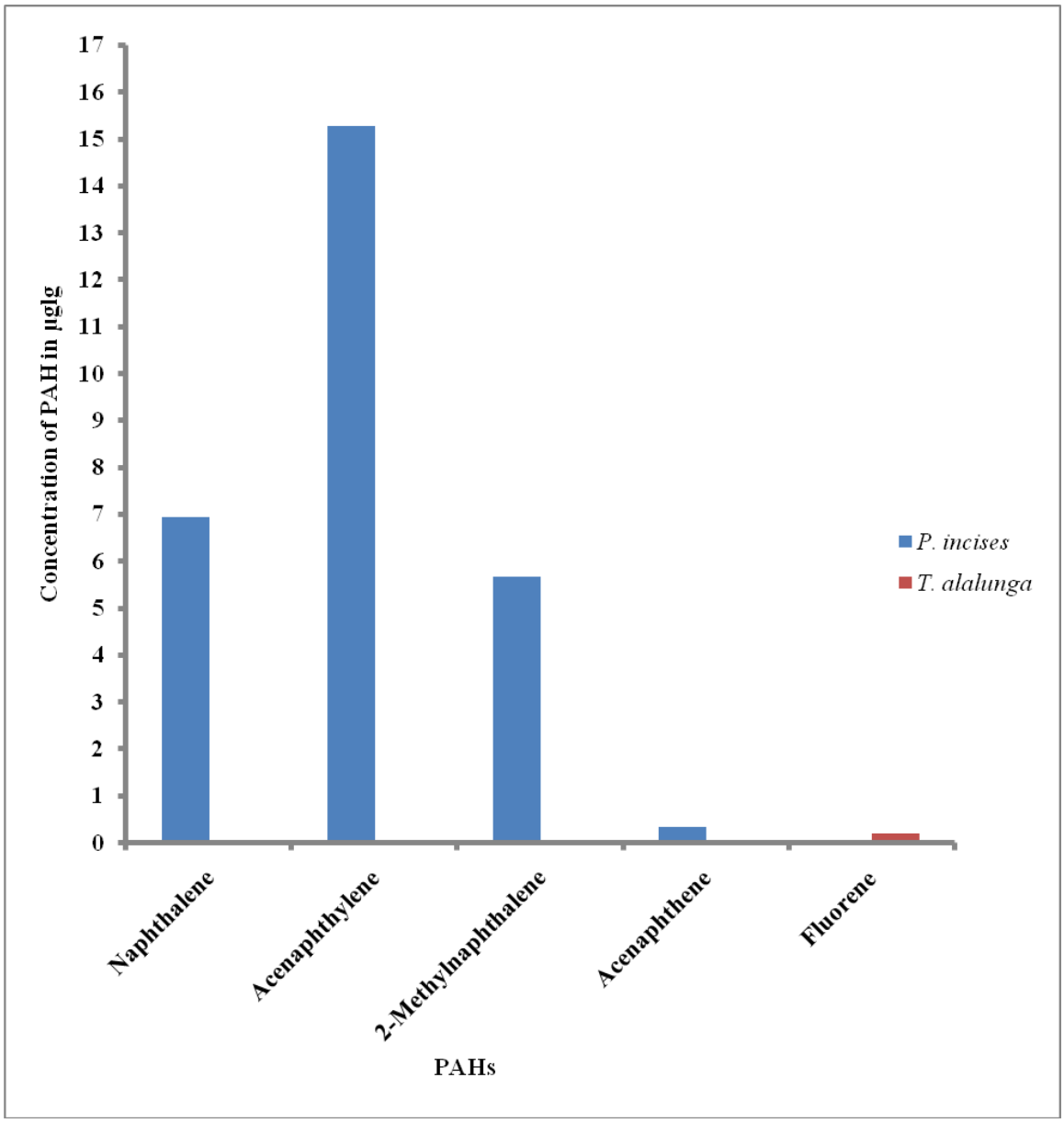

Figure 3. 1 PAH concentrations in *P. Incises* liver from Aboadze in Shama District and *T. alalunga* fish from Dixcove in Ahanta West District

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

3.1.2 PAHs in Water Samples

Inland water bodies including rivers and dug wells used for domestic purposes were analysed for the water PAHs. Samples were collected from four out of the six study sites/ communities. These four communities are Atuabo in Ellembelle, 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 as shown in figure 3.2, naphthalene, acenaphthylene, 1-methlynaphthalene, fluorene, phenanthrene, fluoranthene, chrysene and benz[a]fluoranthene 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 μg/L, respectively. Water bodies from Half Assini, as shown in figure 3.3 had the following PAHs: naphthalene, 1-methlynaphthalene, fluorene, phenanthrene, fluoranthene, 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 μg/L, respectively.

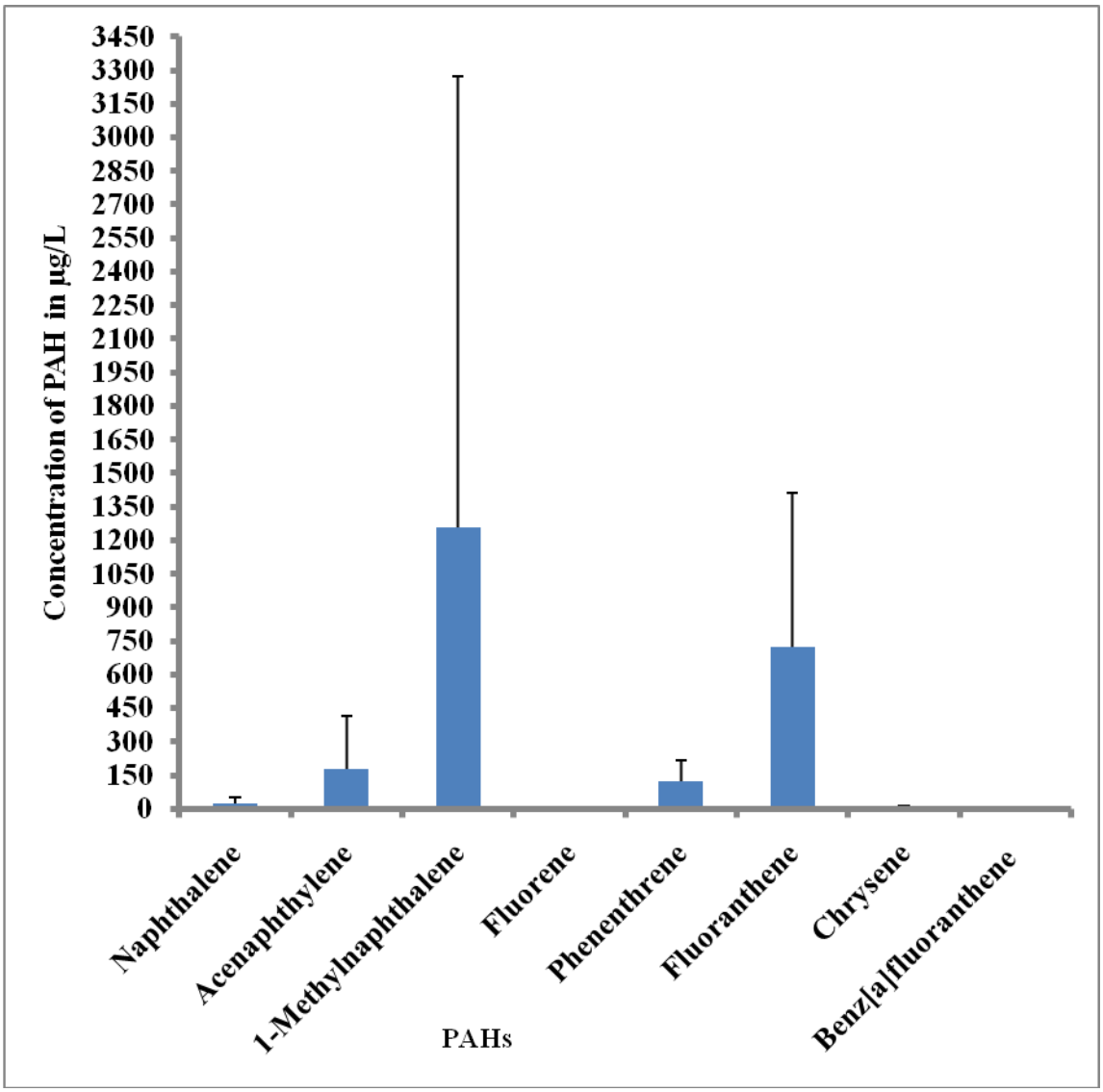

Figure 3. 2 PAH concentration in water samples from Dixcove in Ahanta West District.

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 ± SD. N=5

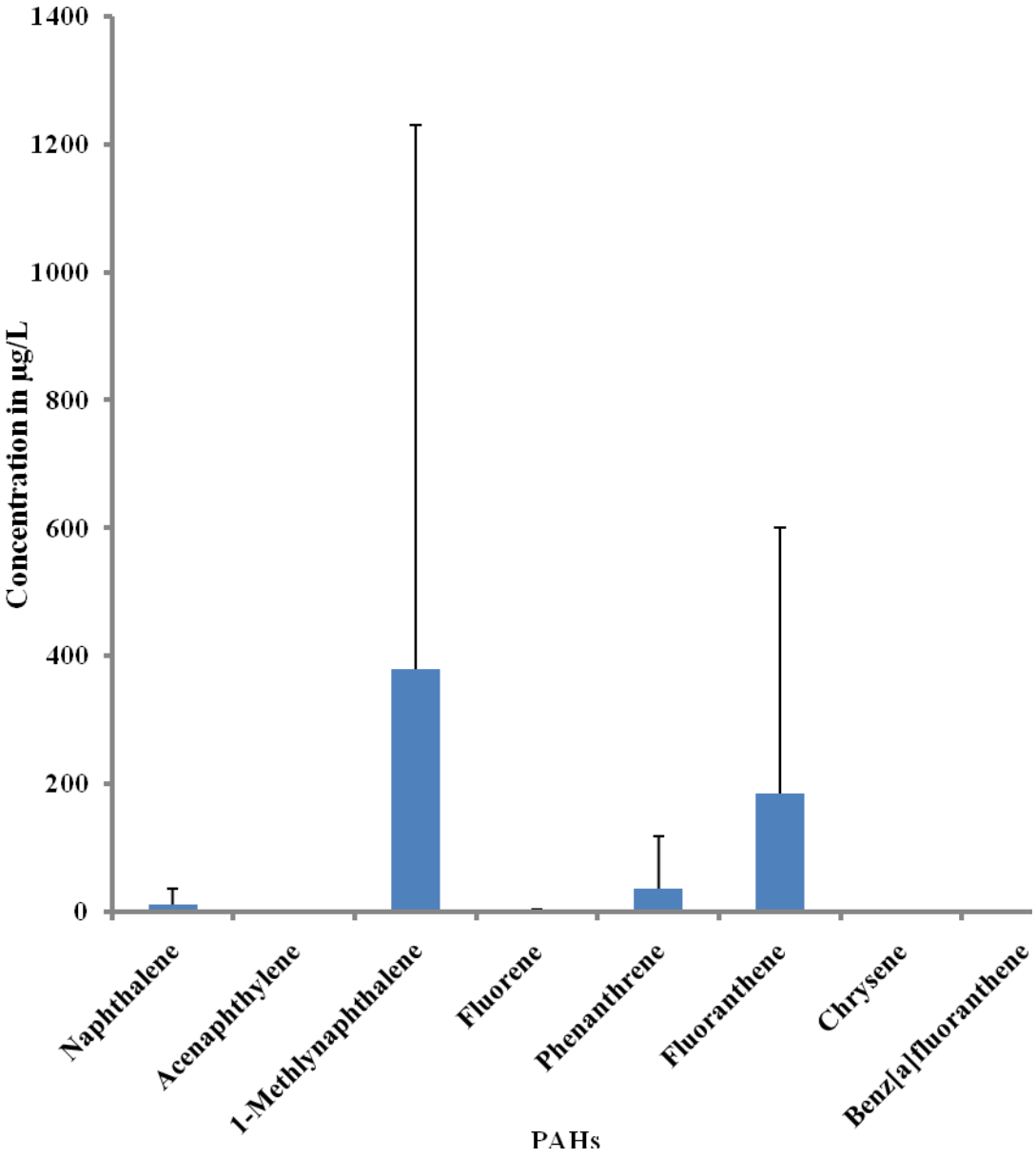

Figure 3. 3 PAHs concentration in water samples from Half Assini in Jomoro District.

Water samples from five water bodies in this study site were subjected to solid phase extraction and before reverse phase HPLC analysis after which concentration of each PAH compound was calculated relative to concentration of PAH standards. Results are means ± SD .N=5.

3.1.3 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, Avicennia nitida, Chromolaena odorata, Erythrina senegalensis, Ficus exasperata, Ficus sagittifolia, Ficus umbellata, Terminalia catappa, Thespesia populnea, Spondias mombim 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.

In all, only plant species *Erythrina senegalensis* and *Ficus umbellata* from Lower Axim in Nzema East District had some PAHs detected. From 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* as can be seen in figure 3.4

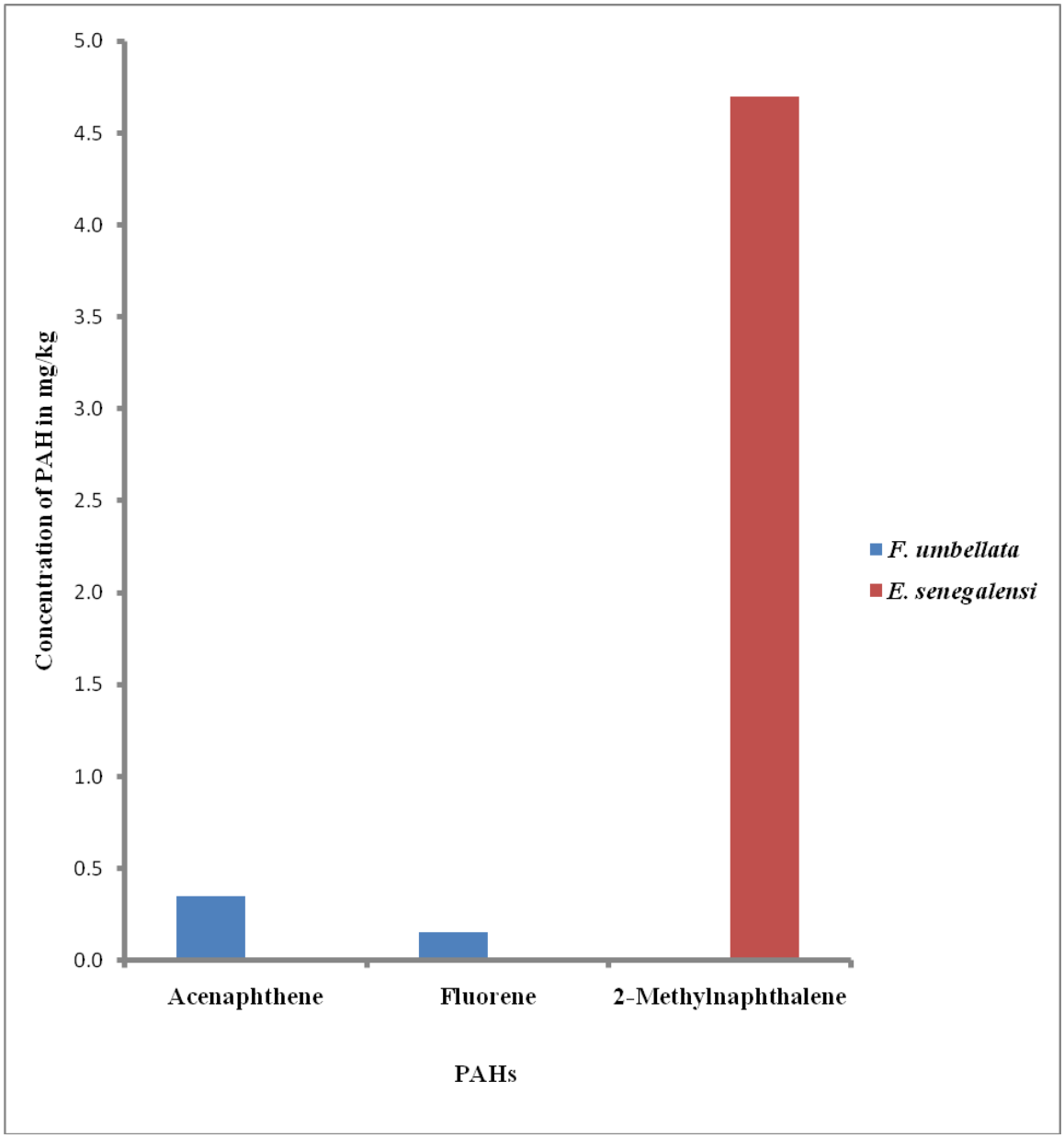

Figure 3. 4 PAHs concentration in plant samples from Lower Axim in Nzema East District.

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

3.1.4 PAHs in Soil Samples

Soil samples were taken from two different depths of 0 – 15 cm, and 15 - 30 cm and used for the analysis in this study. The total number of soil sampled for this study was 60 (composite sampling of 3 different spots per location of 2 different depths). The average concentrations of the various PAHs were computed from all the sampling sites. As shown in Figure 3.5, samples from Aboadze in the Shama district recorded seven PAHs from soil depth of 0 - 15 cm. 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 3.6 shows mean concentrations of PAHs in soil samples from Dixcove in the Ahanta West District. Only Pyrene (0.220 ± 0.492 mg/Kg) was found at depth of 0-15 cm. The compounds (with concentrations at 0-15 cm and 15-30 cm, respectively) were as follows: 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), acenaphthene (0.600 ± 1.475 and 1.100 ± 2.459 mg/Kg) and anthracene (0.700 ± 1.565 and 0.86 ± 1.923 mg/Kg). However, naphthalene (0.220 ± 0.490 mg/Kg), acenaphthylene (12.680 ± 28.350 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.

Soil PAHs levels for Lower Axim in the Nzema East District are shown in Figure 3.7. 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.

In Half Assini of the Jomoro District, PAHs were detected only at depths of between 0 - 15 cm. As shown in figure 3.8, the PAHs were acenaphthene (0.160 ± 0.357 mg/Kg), fluorene (0.100 ± 0.223 mg/Kg), phenanthrene (1.580 ± 0.353 mg/Kg) anthracene (0.200 ± 0.447 mg/Kg) and fluoranthene (95.360 ± 21.322 mg/Kg).

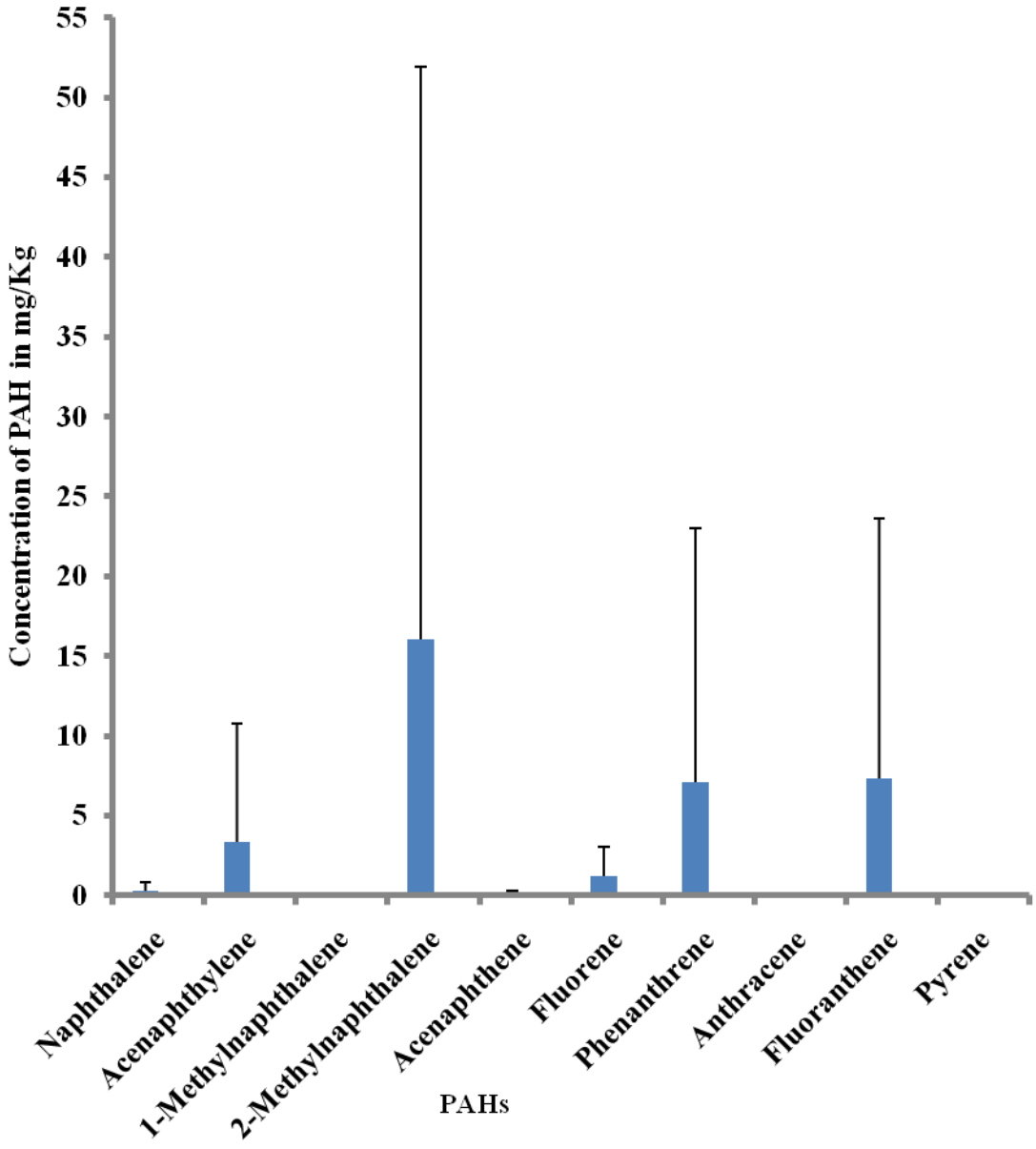

Figure 3. 5 PAHs in soil samples from Aboadze in the Shama District.

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 ± SD. N=5.

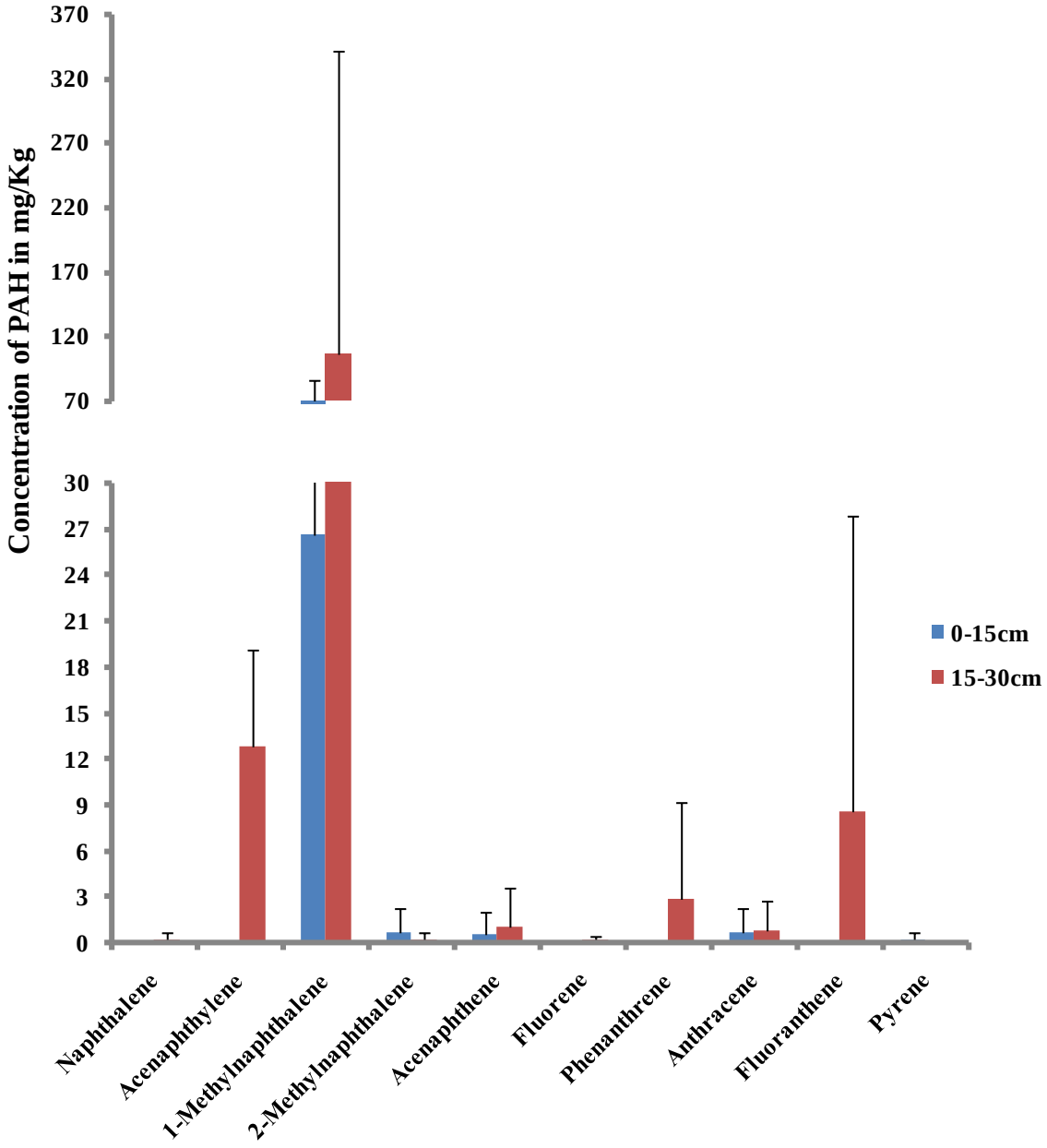

Figure 3. 6 PAHs in soil samples from Dixcove in the Ahanta West District

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. Concentration of each compound was computed relative to concentration of PAH standard. Blue bar-soil taken from 0-15cm. Red bar-soil taken from 15-30cm. Blue bar-soil taken from 0-15cm. Red bar-soil taken from 15-30cm. Red bars indicate soil taken from 15-30cm. Results are means ± SD. N=5.

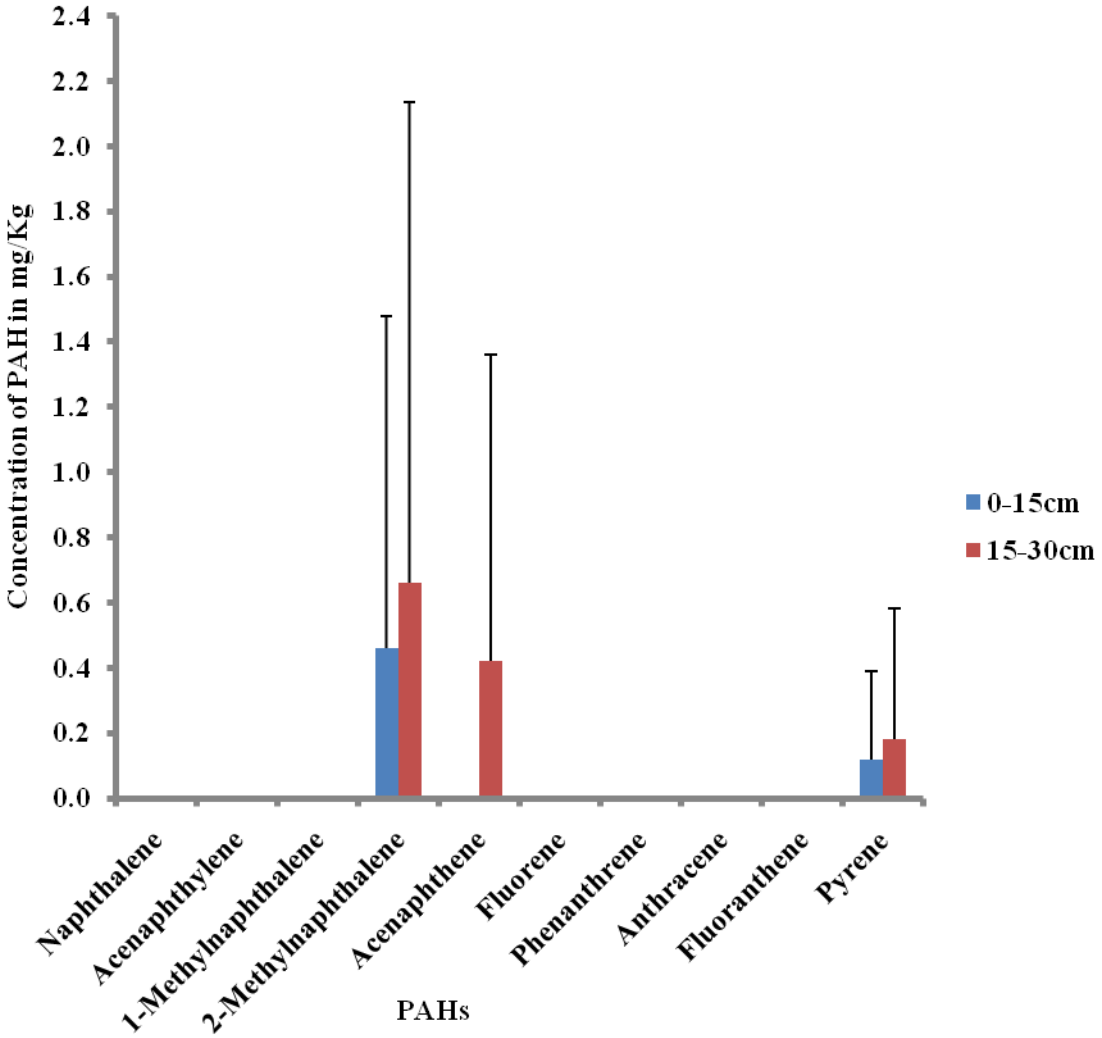

Figure 3. 7 PAHs in soil samples from Lower Axim in the Nzema East District.

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 bar-soil taken from 0-15cm. Red bar-soil taken from 15-30cm. Red bars indicate soil taken from 15-30cm. Results are means ± SD. N=5.

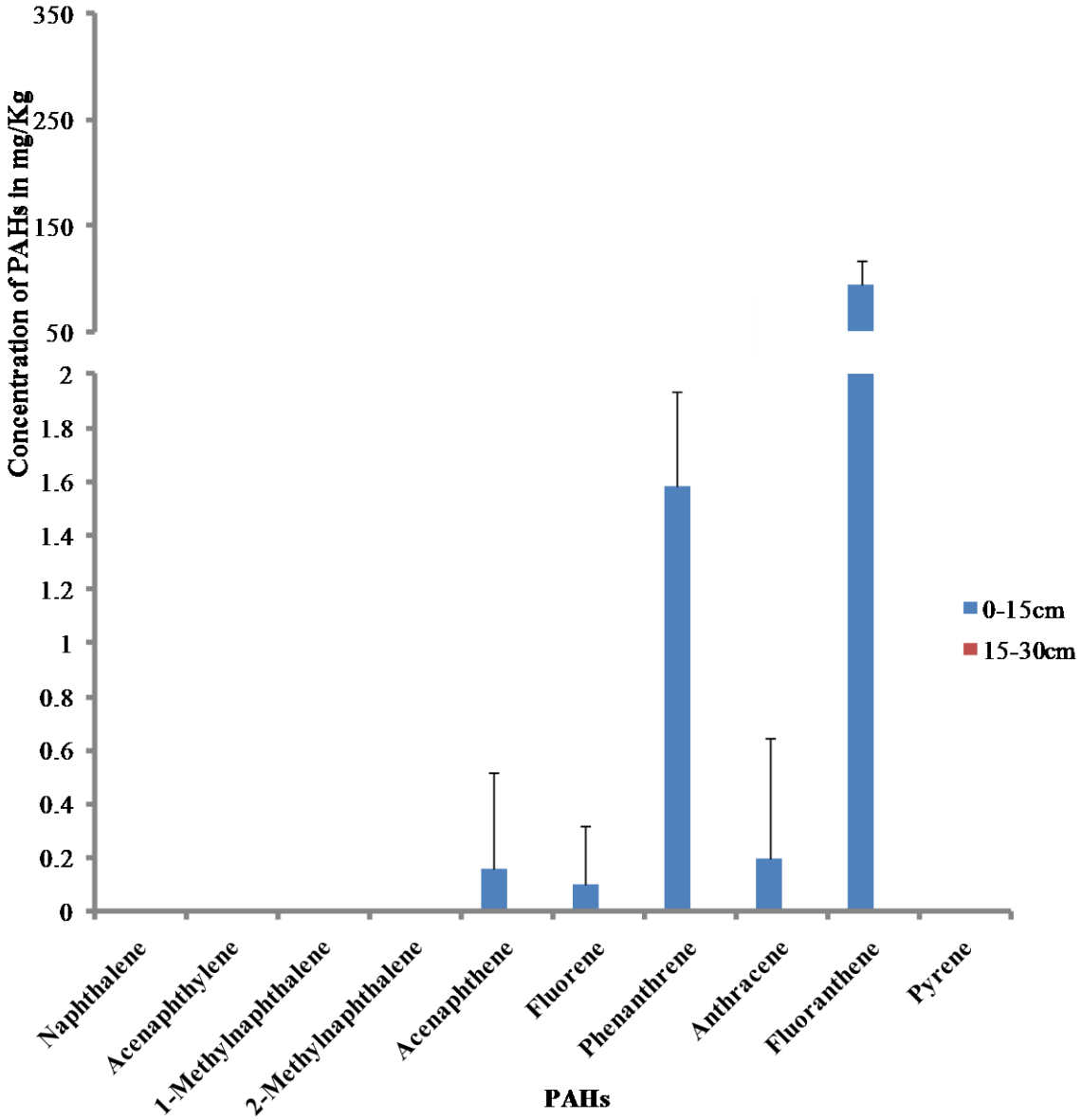

Figure 3. 8 PAHs in soil samples from Half Assini in the Jomoro District.

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 the concentrations of PAH standards. Blue bar-soil taken from 0-15cm. Red bar-soil taken from 15-30cm. Red bars indicate soil taken from 15-30cm. Results are means ± SD. N=5

3.2 Micronuclei in Fish Erythrocytes

Erythrocytes were examined using an optical microscope at 100X magnification. Only cells with intact cellular and nuclear membranes were scored. For each of the three fish of the same species, ten different fields were counted and then averaged as a representative value before being normalized using equation 2.1. The mean micronuclei frequency per 1000 cells and standard deviation 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 and Lower Axim are shown in Figures 3.9 and 3.10, respectively. At Atuabo six fish species, namely *Caranx chrysurus, Seriola dumurili, Ethmalosa dorsalis, Sphyraena sphyraena, Selene dorsalis, Caranx hippos* were analysed for the presence of the micronuclei. Only the three species *Caranx chrysurus, Sphyraena sphyraena and Selene 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 3.9). In Lower Axim (Figure 3.10), all the six fish species, namely *Brachydeuterus auritus, Dentex angolensis, Chloroscombrus chrysurus, Sphyraena sphyraena, Stromateus fiatola and Pseudotolithus 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 *Brachydeuterus auritus, Sphyraena sphyraena, Sardinella aurita, Hemiramphus brasiliensis, Pomadasys incisus and Acanthurus monroviae* from Half Assini, only *B. auritus* recorded MNEs/1000 cells of 2.21 ± 3.38

At Aboadze, these fish species; *Illisha africana, Sardinella eba, Sardinella aurita, Brachydeuterus auritus, Pomadasys incises, Dentex congoensis, Pseudupe prayensis, Lutjanus fulgens and Katsuwonus pelamis* recorded no MNEs. Likewise fish species *Galeoides decadactylus, Pentanemus quinquarius, Pseudotholitus senegalensis, Brachydeuterus auritus* and *Illisha africana* from New-Takoradi landing beach in the Sekondi-Takoradi Metropolitan District had no MNEs. Moreover, in Dixcove of the Ahanta West District, the sampled fish species *Trichiurus lepturus, Thunnus albacores, Katsuwonus pelamis and Thunnus alalunga*, recorded no MNEs.

Table 3. 1: 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
Brachydeuterus auritus	2.21	3.83	6.64	0.00
Sphyraena sphyraena	ND	ND	ND	ND
Sardinella aurita	ND	ND	ND	ND
Hemiramphus brasiliensis	ND	ND	ND	ND
Pomadasys incisus	ND	ND	ND	ND
Acanthurus monroviae	ND	ND	ND	ND

Thin smears of peripheral blood from three fish per six different fish species were stained with Giemsa and Maygrunnwald solutions and then cells observed under a light microscope at × 100 magnifications for ten fields of each slide prepared. Data is presented as the mean frequency of micronuclei ± SD of N =3. ND means not detected. MN-Micronuclei, SD-Standard Deviation, MAX-Maximum, MIN-Minimum

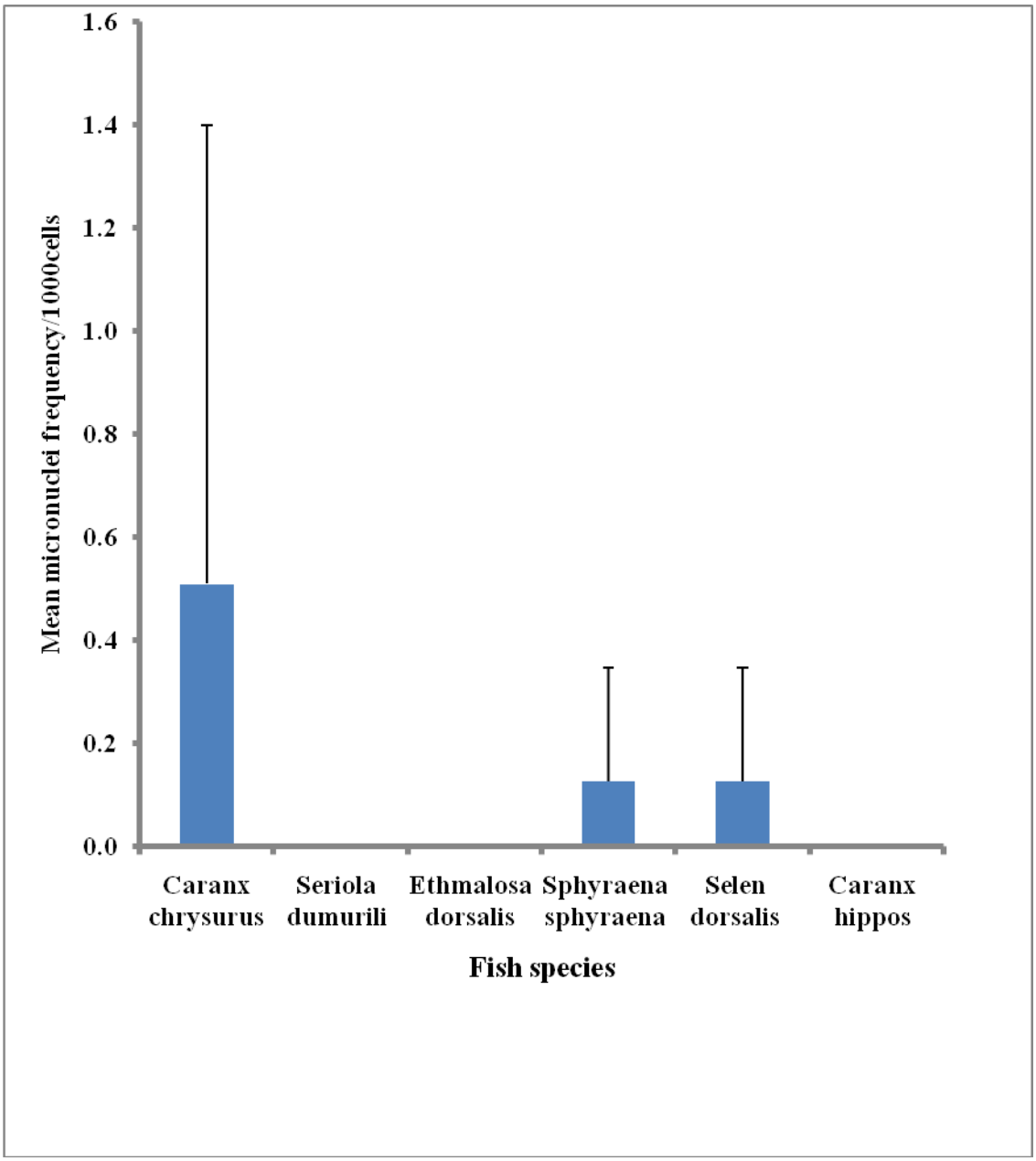

Figure 3. 9 Mean micronuclei observed in fish species from Atuabo in Ellembelle District.

Thin smears of peripheral blood from three fish per six different fish species were stained with Giemsa and Maygrunnwald solutions and then cells observed under a light microscope at × 100 magnifications for ten fields of each slide prepared. Data is presented as the mean frequency of micronuclei ± SD of N =3.

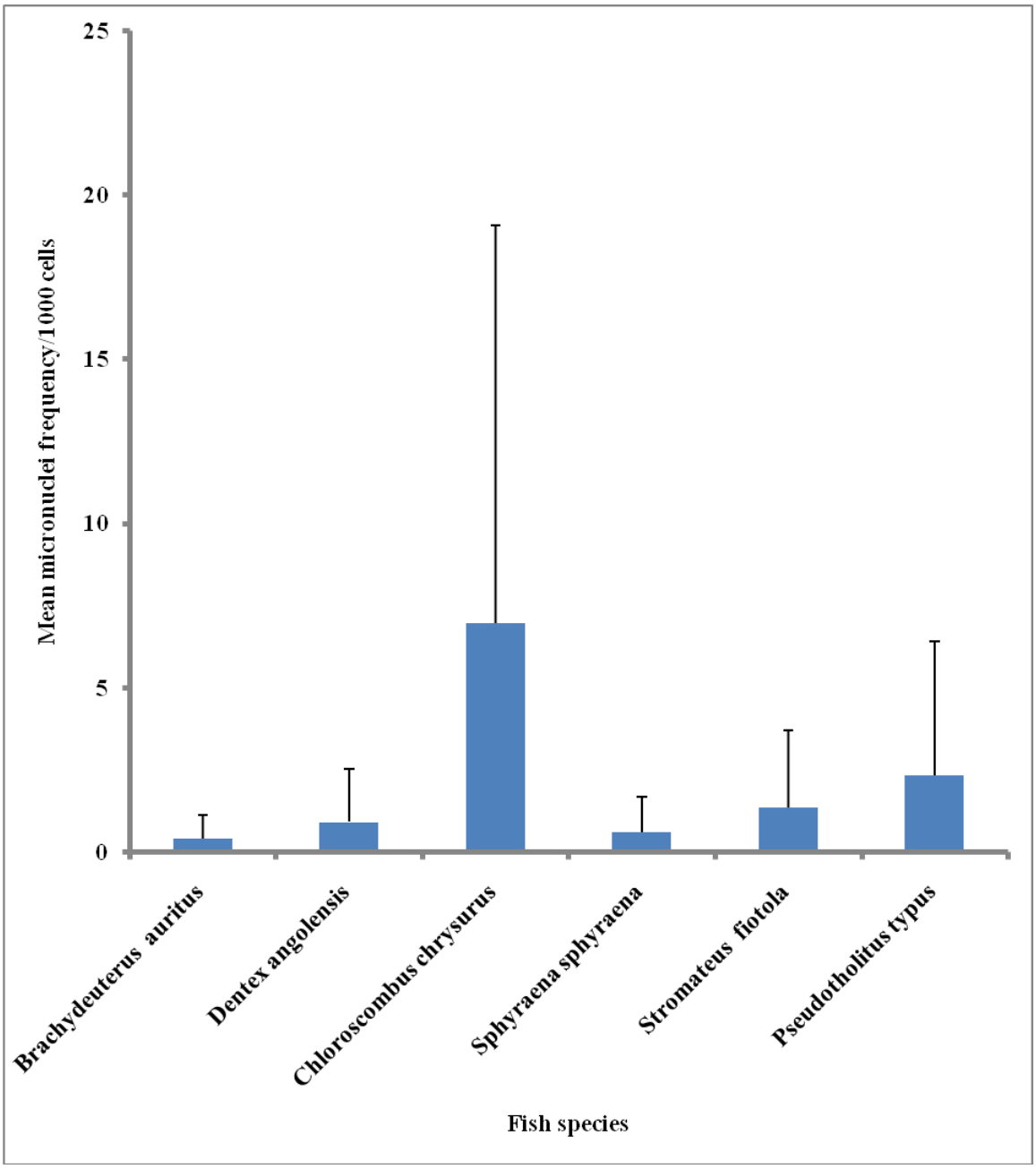

Figure 3. 10 Mean micronuclei counts in different fish species from Lower Axim in Nzema East District.

Thin smears of peripheral blood from three fish per six different fish species were stained with Giemsa and Maygrunnwald solutions and then cells observed under a light microscope at × 100 magnifications for ten fields of each slide prepared. Data is presented as the mean frequency of micronuclei ± SD. N=3.

CHAPTER FOUR

4.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 DISCUSSION

The environment can be polluted in various ways through oil drilling activities, industry, land reclamation, erosion and the continuous urbanization. Most often, environmental pollution induced through these means could have adverse consequences for human health when humans are exposed to high levels of such toxicants that may pollute the air, plants, animals and water bodies. These toxic substances are able to persist in the environment because water bodies and land (with their fauna and flora), play a role in harbouring, retaining and depositing these toxic substances (Li *et al.*, 2009). The volatile components of the toxic substances are also able to contaminate atmospheric air. The most frequent contaminants of the environment, especially surrounding oil drilling fields are oil spills, mineral oil and polycyclic aromatic hydrocarbons (PAHs). Actually, there are over hundreds of PAHs however, the group of PAHs that pose health risks or impacts generally includes: naphthalene, acenaphthylene, 1-methylnaphthalene, 2-methylnaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benz[a]fluoranthene and benz[k]fluoranthene. In this study, baseline assessment of biomarkers of pollution resulting from oil drilling of the Jubilee Oil Fields in the Western region of Ghana was assessed. DNA adducts formation in fish samples and the presences of PAHs were of particular interest as biomarkers of pollution.

The presence of high levels of PAHs in fish tissues is suggestive of PAH contamination of coastal water from sources which could include oil slicks, accidental discharges of oil from ships and fishing boats, discharges from the crude oil drilling site, dumping of domestic wastes on the coast, and smoke emissions from coastal industries (Neff and Burns, 1996). Fishes are good model organisms for detecting the presence of pollutants in the aquatic environment because of their sensitivity to low concentrations of genotoxic substances (Al-Sabtiand Metcalfe, 1995). Fishes take up these toxicants, bioconcentrate them in organs such as gills, liver and to a lesser extent muscle tissues (Baussant *et al*, 2001). Due to the hydrophobic nature of PAHs, they tend to accumulate in the fatty tissues of fish following their uptake (Bouloubassi *et al*., 2001). The lipophilic nature of PAHs affect their distribution in water column, thus in most cases high and low molecular weight PAHs form sediments from the water column by particles that tend to settle at the bottom of water bodies particles and volatilization (Neff and Burns, 1996). It would be expected that analysis of pelagic fishes, which are surface water dwellers would indicate the absence of high molecular weight PAHs in their tissues or even if they are present their concentration will be very low. In this study, a number of fish species both pelagic and demersal, from each of the six study sites were analyzed for PAHs in their tissues and the results indicated the presence of PAHs in both pelagic and demersal fish samples. The results as presented in figure 3.1 indicate the presence of PAHs in demersal fish species *P. Incises*. The PAHs detected in this species include naphthalene, acenaphthylene, 2-methylnaphthalene and acenaphthene all of which are low molecular weight PAHs. Their uptake by this particular demersal species may be due to the presence of these PAHs in high concentrations in sediments where this species dwells.

According to Saha *et al*.(2009), the predominance of lower molecular weight PAHs points to petrogenic sources; thus the presence of these low molecular weight PAHs in *P. incises* can be attributed to petrogenic sources of which which include crude oil drilling activities. Fluorene was the only PAH detected in the pelagic fish species *T. alalunga* caught from Dixcove. Fluorene, is one of the low molecular weight PAHs and thus its presence in this specie is expected when their habitat is contaminated by PAHs. The absence of PAH in fish which had 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 these findings depict very low levels of PAHs, such low concentration of contaminants can trigger the genotoxicity effects of PAHs in fish and by extension in coastal dwellers (White, 2002). However, higher degrees of toxicity are encountered with exposure to higher levels of PAHs. PAHs concentrations above the threshold limit value of 30 µg/Kg are deemed to be toxic (US-EPA, 2000). The consumption of fish is very popular in Ghana as it constitutes a major source of animal protein in the diet (Asamoah *et al*., 2012). People living in coastal communities 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), endocrine alteration, malformations of embryo, DNA damage and birth defects (Choi *et al*., 2006).

The aquatic environment receives influx of PAH from two major sources: the movement of water containing dissolved and particulate constituents derived from watersheds; and atmospheric deposition both in precipitation and dry deposition from airsheds of coastal

ocean (Latimer and Zheng, 2003). In addition to these major sources, oil spills and leaks during the transport and production of petroleum also account for significant levels of PAHs in coastal marine environment (Stout and Wang, 2007).

Currently, petroleum sources are the dominant energy source in most countries of which Ghana is included and it is expected to remain so over the next several decades (Kharaka and Dorsey, 2005). Thus, there is a guaranteed basis for the continued release of PAHs into the environment. Baseline assessment of PAHs in water bodies used for domestic purposes in these study areas revealed the presence of fluoranthene, phenanthrene, fluorene naphthalene and its methylated form 1-methylnaphthalene in the water sources in Dixcove. Of the various PAHs measured in water for regulatory purposes, fluoranthene is the only PAH that was detected to a significant extent.

According to the World Health Organization, concentrations of individual PAHs in water bodies are generally found to be 50 ng/L and hence any concentration above this level indicates rather toxic levels (WHO, 2008). The source of contamination could be through industrial point sources and shipyards, atmospheric deposition, and urban runoff and drilling activities as anticipated in this study. It must be pointed out ,however that the study sites with higher risk of being polluted by virtue of their closeness to the drilling sites, harbor no manufacturing industries (they are mainly coastal fishing communities) thus leaching and atmospheric deposition may account for the elevated levels of fluoranthene recorded particularly in Dixcove. Leaching of PAH in water bodies has been demonstrated to be the primary route for contamination of water bodies as the

compounds tend to be strongly adsorbed on soil organic matter (Oros *et al*., 2007). There are reports on 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 obtained in this study compares with other results on investigations carried out to find the level of PAH contaminations in water bodies from England and Wales where PAH levels above the set standard of 0.2 µg/l in water bodies were recorded (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, 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 presence of PAHs because they are considered as important sinks for atmospheric PAHs and are involved in the periodic cycling of PAHs (Slaski *et al.*, 2000; Zygmunt and Namiesnik, 2003). Studies have demonstrated the use of plants as a quantitative indicator of exposure to both gaseous and solid phase PAHs in the environment (Korury *et al*., 1999; Zare-Maivan, 2011). The leaves of plants in particular have been identified as the primary sinks of airborne PAH compounds, however absorption of PAHs from soils through the root system is very minimal (De Nicola *et al*., 2011). Of all the leaf samples collected from the study sites, PAHs were

detected in only two species, *Erythrina senegalensis and Ficus umbellata* from Lower Axim, which were among the dominant species from the various sampling clusters in the study sites. These plants species include *Alchornea cordifolia, Avicennia nitida, Chromolaena odorata, Erythrina senegalensis, Ficus exasperata, Ficus sagittifolia, Ficus umbellata, Terminalia catappa, Thespesia populnea, Senna siamea , Spondias mombim* and *Terminalia catappa*. Thus, for future monitoring of PAHs, the plant species *Erythrina senegalensis* and *Ficus umbellata* can be used as a good indicators for evaluation of PAH pollution. 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, 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 are suggestive of 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 1-methylnaphthalene, 2-methylnaphthalene, acenaphthene and anthracene were found in the different soil depths from Dixcove in the Ahanta West

District. However, naphthalene, acenaphthylene, fluorene and phenanthrene were only found at depths of 15 to 30 cm which is suggestive of leaching of these compounds to this depth. The source of these volatile PAHs in this deep soil depth may therefore be attributed to petrogenic sources. The only high molecular weight PAH compound found in Dixcove was pyrene and this was detected in the 0 - 15 cm depth.

With respect to Lower Axim, 2-methylnaphthalene, acenaphthene and pyrene were the only PAH compounds detected. Both 2-methylnaphthalene and acenaphthene are low molecular weight PAHs that were found at the depth of 15-30 cm with 2-methylnaphthalene also detected at the depth of 0-15 cm. Pyrene, which is the only high molecular weight PAH found in Lower Axim was also detected at both depths (surface and deep). At Half Assini, acenaphthene, fluorene, phenanthrene, anthracene and fluoranthene were the PAHs detected with all except fluoranthene being detected at a depth of 0-15 cm. Fluoranthene which is the only high molecular weight PAH detected in this town was found at both depths. Dixcove and Lower Axim are coastal towns which are closer 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 and these were detected at a depth of 0-15 cm. But none of these low molecular weight PAHs had leached to a deeper depth or probably the concentrations at this depth were too low to be detected. Aboadze is a coastal town which is far from the drilling site, but has a thermal plant belonging to the Volta River Authority (VRA). Thermal plants burn diesel, which is a component of crude oil to generate electricity thus the PAH compounds found 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 naphthalene, acenaphthylene and acenaphthene on soil surfaces (depth of 0-15cm). It is also possible that activities in the area have so far not generated any detectable levels of PAHs. The results revealed fluoranthene and 1-methylnaphthalene as being the compounds with high concentrations in soil samples from Half Assini and Dixcove.

According to De Lemos *et al*. (2008), a frequency of over 15% micronucleus in peripheral erythrocytes of an organism provides an indication of the presence of genotoxic agents such as PAH in the environment. Thus, a baseline and periodic assessment of this biomarker is imperative to regulate the release of these chemical pollutants into the aquatic environment and also initiate bioremediation process to curtail the potential toxic effects. The micronucleus assay can, therefore, be adopted for bio-monitoring as it is a fast and sensitive indicator of structural alterations, DNA loss, and numerical chromosomal abnormalities (Galindo and Moreira, 2009). In this environmental study, different fish species obtained from the communities bordering the oil drilling field were employed for the establishment of 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 Jomoro district in which 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 is quite similar to results found for *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 analysed including the mean of *C. chrysurus* from Lower Axim which showed the highest levels (6.98 ± 12.09) . Though these results suggest very minimal contamination, continuous monitoring should be done since any change in the environment may be easily detected by an increase in these frequencies. On the other hand, micronuclei were observed in peripheral erythrocytes from fish species caught in the communities close to the drilling sites but they were also of no statistical significance when analysed by one way ANOVA at a p value < 0.05. Results from other studies indicate a very low range of MNE frequency and also show a large range of variability in the frequency from 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 the frequency of nuclear anomalies and the exposure to genotoxic agents is well established (Al-Sabti and Metcalfe, 1995).

4.2 CONCLUSION

This study has provided baseline data for assessing pollution of six districts near by 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. 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 be indicative of 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 analysed.

4.3 RECOMMENDATIONS

I recommend that periodic monitoring of these communities should be done with reference to these baseline values to monitor the levels of pollution resulting from oil drilling and avoid adverse effects to the communities living close to the oil fields.

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APPENDICES

Table A. 1: List of fish species used for this study

Study sites	Local name	Scientific name
Ellembelle/Atuabo	Anoeku	*Caranx chrysurus*
	Mbroma/Abedipiatakaw	*Seriola dumurili*
	Kokora/Ngokolo	*Ethmalosa dorsalis*
	Eel	*Sphyraena sphyraena*
	Antele wawec	*Selene dorsalis*
	NI	*Caranx hippos*
Jomorro/Half Assini	Eboe	*Brachydeuterus auritus*
	Edoe/Eloe	*Sphyraena sphyraena*
	Sardines	*Sardinella aurita*
	Daye	*Hemiramphus brasiliensis*
	Kwakuwa	*Pomadasys incisus*
	Alata blade	*Acanthurus monroviae*
Nzema East/Lower Axim	Eboe	*Brachydeuterus auritus*
	Sikasika	*Dentex angolensis*
	NI	*Chloroscombrus chrysurus*
	Tantamire	*Sphyraena Sphyraena*
	Ahorlorlor/Mamaniwa	*Stromateus fiotola*
	Awomakpoke	*Pseudotholithus typus*
AhantaWest/Dixcove	Waewayan	*Trichiurus lepturus*
	Edei	*Thunnus albacares*
	Anful	*Katsuwonus pelamis*
	Okatae	*NI*
	Kwabui	*NI*
	Edae	*Thunnus alalunga*

NI means Not Identified

Table A. 2: List of fish species used for this study.

Sampling sites	Local name	Scientific name
Shama	Nkafona	*Illisha Africana*
	Herring	*Sardinella eba*
	Sardines	*Sardinella aurita*
	Eboe	*Brachydeuterus auritus*
	Kwakuwa	*Pomadasys incisus*
	Wereweru	*Dentex congoensis*
	Kukurudy	*Pseudupe prayensis*
	Esopei	*Lutjanus fulgens*
	Tuna	*Katsuwonus pelamis*
Sekondi-Takoradi	Sukpui	*Galeoides decadactyius*
	Ankasa-ankasa	*Pentanemus quinquarius*
	Cassava fish	*Pseudotholitus senegalensis*
	Eboe	*Brachydeuterus auritus*
	Nkafona	*Illisha Africana*

Table A. 3: List of plant species collected from the study sites.

Sampling clusters	Study sites		
	Aboadze	New Takoradi	Dixcove
1	*Avecinia nitida* *Canavalia sp*	*Cassia siamea* *Sida cordifolia*	*Chromolaena odorata* *Justicia flava*
2	*Luffa aegyptiaca* *Indigofera arrecta*	*Phyllanthus urinaria* *Ipomea pes caprae*	*Vigna sp* *Hypselodelphys violacea*
3	*Cassia siamea* *Chromolaena odorata*	*Cassia siamea* *Samanea saman*	*Combretum racemosum* *Phyllanthus urinaria*
4	*Sacrocephalus glabra* *Griffonia simplicifolia*	*Cassia siamea* *Ficus umbellata*	*Cassia siamea* *Pueraria phaseoloides*
5	*Cassia siamea* *Morinda lucida*	*Ficus exasperata* *Cassia siamea*	*Ficus umbellata* *Benthi sp*

Table A. 4: List of plant species collected from the study sites.

Sampling clusters	Study sites		
	Lower Axim	Atuabo	Half Assini
1	*Erythrina senegalensis* *Spondias mombim*	NI (EA1A) *Aframomum elliotii*	*Cocos nucifera* *Terminalia catappa*
2	*Spondias mombim* *Erythrina senegalensis*	*Smilax kraussiana* *Alchonea cordifolia*	NI (JH2A) *Thespesia populnea*
3	*Ficus umbellata* *Erythrina senegalensis*	*Ficus sagittifolia* *Thespesia populnea*	*Thespesia populnea* *Gmelina arborea,*
4	*Ficus umbellata* *Terminalia catapa*	*Coccoloba uvifera* *Cocos nucifera*	*Alchornea cordifolia* *Coccoloba uvifera*
5	*Ficus umbellata* *Erythrina senegalensis*	NI (EA5A) *Ficus elastica*	*Alchornea cordifolia* *Anacardium occidentalis*

NI = not identified, EA1A and EA5A = plant samples collected from Atuabo in the Ellembelle District at sampling locations 1 and 5 respectively whiles JH2A = plants from Half Assini in Jomoro at sampling location 2.

Table A5: Sampling location of six districts bordering the marine coastal environment in the Western Region of Ghana

No	District Name	Study area	land area km^2*	Latitude (N)	Longitude (W)
1	Shama	Aboadze	215.00	4° 580” - 4° 615”	1°.370"-1°.386"
2	Sekondi-Takoradi	New Takoradi	49.78	4° 540” - 4° 545”	1°.119"-1°.450"
3	Ahanta West	Dixcove	591.00	4° 475” - 4° 494”	1°.570"-1°.948"
4	Nzema East	Lower Axim	2194.00	4° 862” - 4° 865”	2°.236"-2°.243”
5	Ellembelle	Atuabo	1468.00	4° 980” - 4° 985”	2°.553"-2°.558"
6	Jomoro	Half Assini	1344.00	5° 048” - 5° 072”	2°.873"-2°.889"

* (Ghana Statistical Service, 2012).

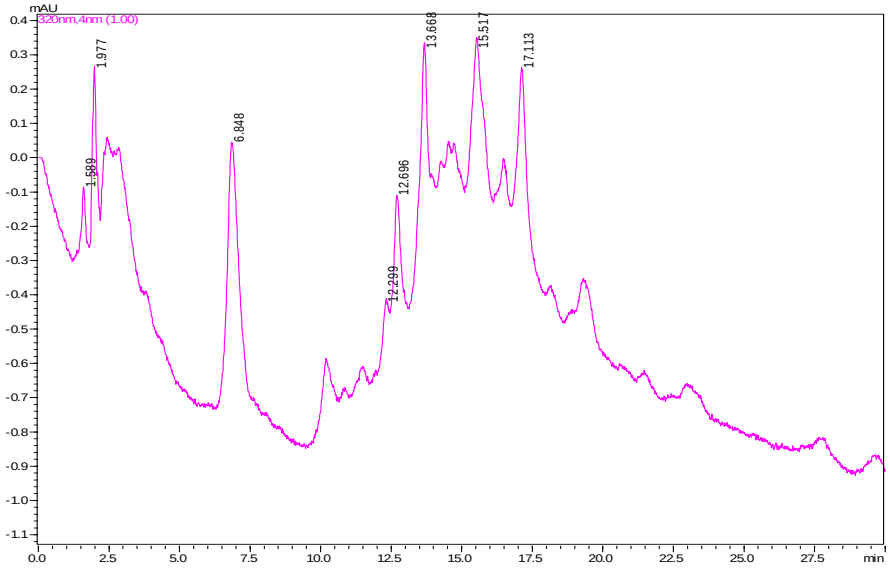

Figure A. 1 Chromatogram of a soil sample (AD1A 0-15) from Dixcove

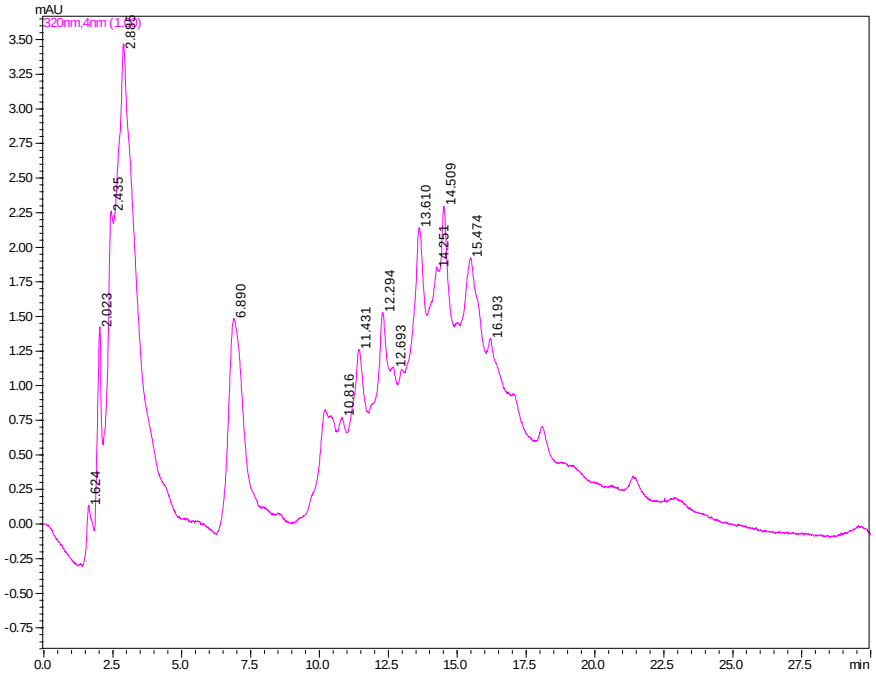

Figure A. 2 Chromatogram of a soil sample (AD1A 15-30) from Dixcove

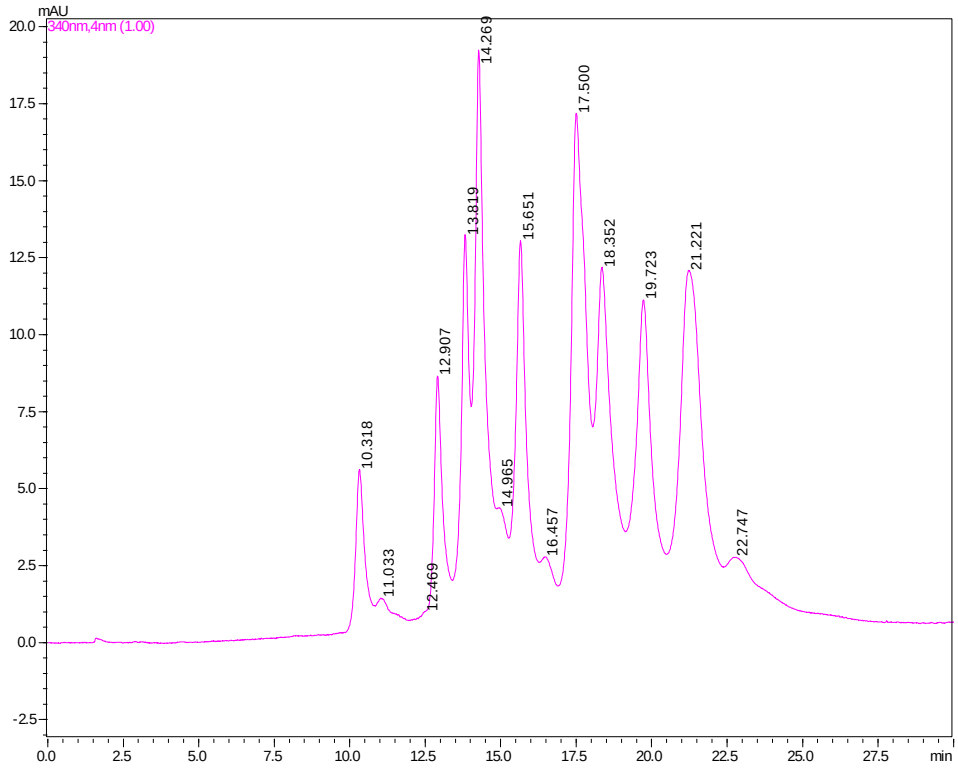

Figure A. 3 Chromatogram of the PAH standard mixture used.

The peaks represent individual PAH compounds with their retention time

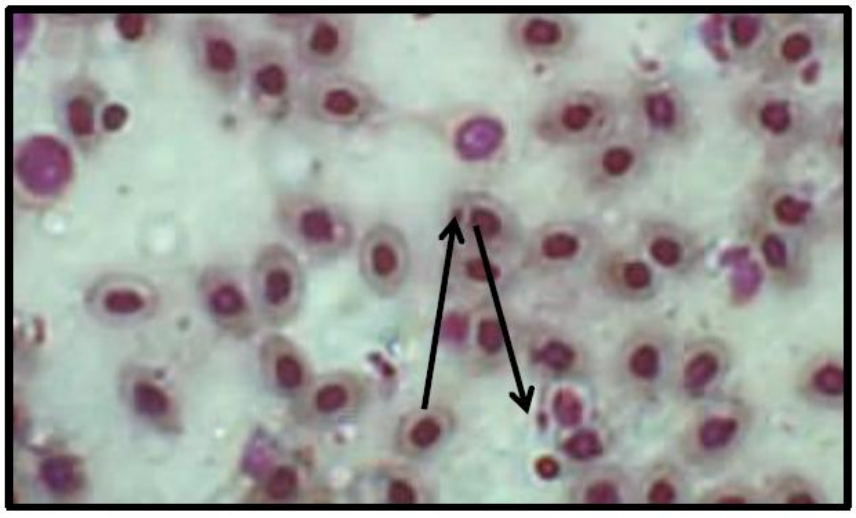

Figure A.4 Micronucleated cells in erythrocytes of *Chloroscombrus chrysurus* (Arrowed).