ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBON (PAH) DISTRIBUTION IN SEDIMENT AND WATER SAMPLES OF WINAM GULF, LAKE VICTORIA, KENYA.

BY

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Protection Agency (USEPA) priority pollutant list. The variation of the detected PAH with season and distance inshore was also investigated. Sampling for sediment samples was done with a surface grab sampler while water undersurface sampling was done with an undersurface sampler. Extraction was according to United Nations environmental Programme protocol number **20,** 1995while purification was according to Christopher et al., 1995. Analysis was done with GC-FID and results confirmed with GC-MS.

The sixteen target PAH were detected in the sediment and water samples, with concentrations in the range of 0.04 to 16.46 μg/g for sediment samples and 3.32 to 55.8 μg/L for water samples, which is an indication of significant pollution of the Gulf by individual PAH. Sediment samples were found to contain relatively higher concentrations of PAH than water samples. The mean concentrations were found to exhibit significant differences ($P \leq 0.05$) with seasons and depths, however the degree of significance could not be established in the cases since only two means were compared. The total amount of individual PAH detected in wet season samples was higher than those detected in dry season samples. The study also shows that the total PAH concentrations in sediment and water samples and the distribution of individual PAH in the samples were different. Water samples predominantly accumulated the lower molecular weight PAH; while sediments accumulated the higher molecular weight PAH.

The total amount of PAH detected in sediments samples taken 5m from the shore was much higher than those detected in sediment samples taken 1m from the shore. Similarly,

the total amount of PAH detected in water samples taken 5m from the shore was less than those detected in water samples taken 10m from the shore. There was also significant differences for the distance and seasonal interactions. Most lower molecular weight PAH like naphthalene and acenaphthylene only showed significant differences 1m from the shore and not 5m from the shore for sediment samples, even with changes in seasons. Most higher molecular weight PAH like Chrysene and pyrene showed significant differences at 5m distances from the shore with seasonal variations. In the water samples, the lower molecular PAH such as acenaphthylene and acenaphthene did not show significant differences with distance, even with seasonal variations. This was also observed with higher molecular weight PAH like benzo(a)anthracene, chrysene and benzo(ghi)perylene.

The average individual PAH accumulation levels in the Car Wash water samples were found to be several magnitudes above the internationally recommended exposure levels. The detected levels were above the Environmental Protection Agency (EPA) carcinogenic accumulation level per kg human body weight for anthracene, acenaphthene, fluoranthene, fluorine and pyrene..

This study therefore showed that Winam Gulf of Lake Victoria is significantly polluted with the investigated PAH. It has also showed that the pollution of the Gulf by the PAH is dependent on season and the distance inshore. This being the first report for this region, the information contained can be vital to environmental conservation organizations and to Kisumu City planners since the City is probably the highest contributor to this pollution.

CHAPTER 1

1.0 INTRODUCTION

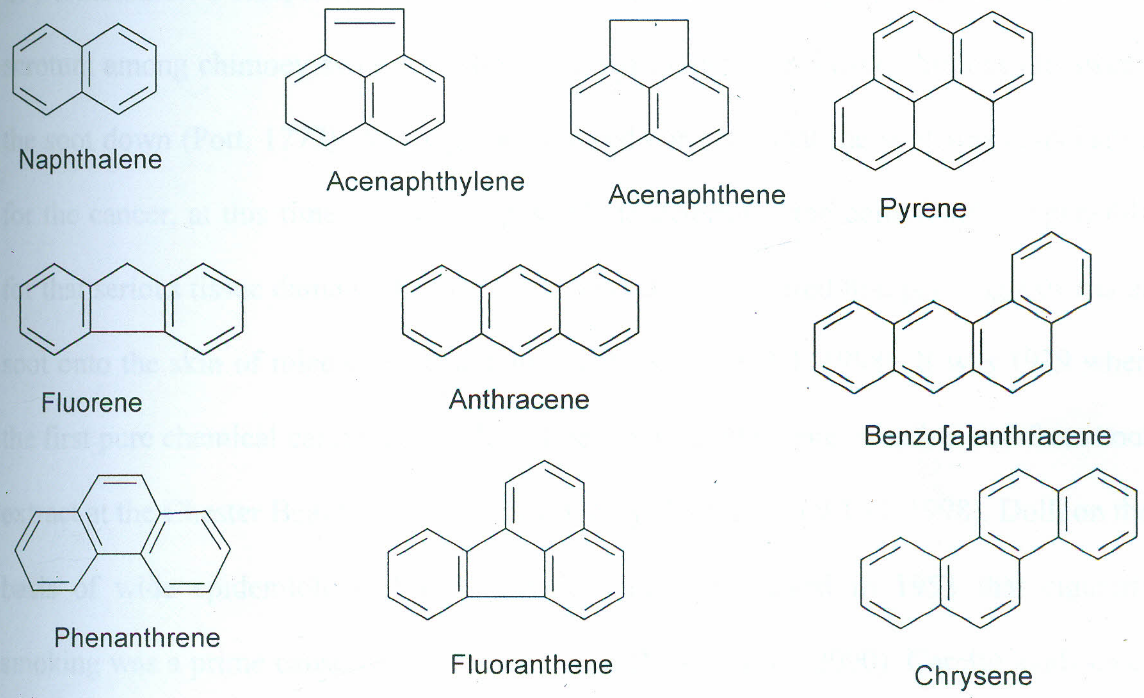
1.1 General

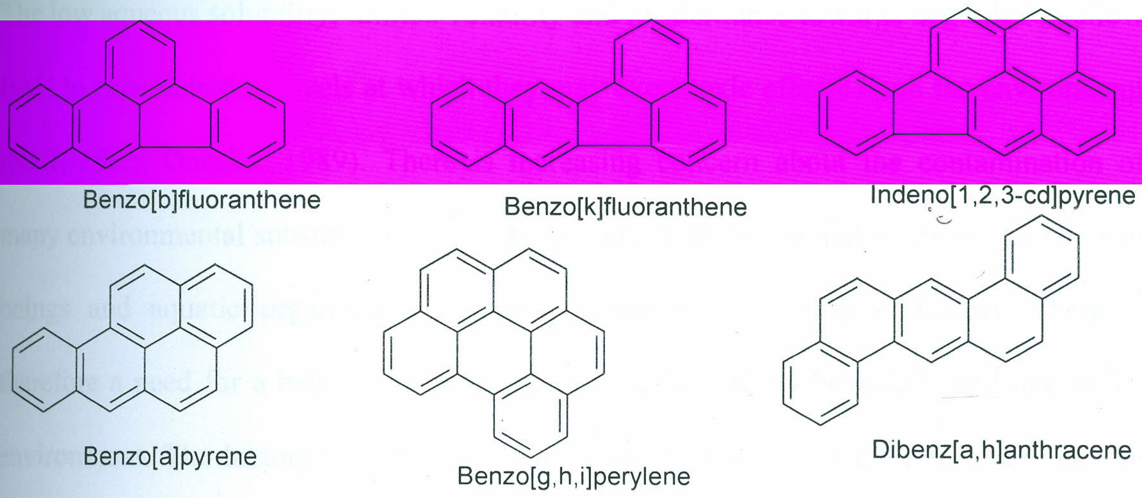
The term polycyclic aromatic hydrocarbon, (PAH), commonly refers to a large class of organic compounds containing two or more fused aromatic rings. In particular, PAH refers to compounds containing carbon and hydrogen atoms (unsubstituted parent PAH and their alkyl-substituted derivatives). They are colourless, white, pale yellow or green solids with melting and boiling points generally above 100^0C (WHO, 1998). They have their origin in both natural and anthropogenic processes. However several studies have indicated that the anthropogenic input of PAH to aquatic sediments far exceeds that of natural sources (NAS, 1971).

Major human activities which produce PAH include pyrolysis of wood to produce charcoal and carbon black, coke production, manufacturing of gas fuel, power generation from fossil fuels, combustion of fuels in internal combustion engines, incineration of industrial and domestic wastes, oil refinery processes and chemical engineering operations among others (Lipkea, 1978; Longwell, 1983; Olten and Senkan, 1999; Weilmuenster *et al.*, 1999; Hall Roberts *et al.*, 2000). By-products of these processes, which contain significant amount of PAH, have been dumped on the land, in the waters or buried at subsurface sites. Airborne particulates, generated from these processes and carrying PAH, are transported worldwide in the atmosphere and usually find their final destination in soils and in sediments of aquatic systems (McVeety and Hites, 1988;

Lipiatou and Albaiges, 1994; Gogou *et al.*, 1996; Wakeham, 1996; Christensen *et al.*, 1997).

PAH are of concern in the environment not only because they are ubiquitous contaminants and recalcitrant towards degradation (NRC, 1983; Lockhart *et al.*, 1992), but also because several members of this class of compounds are well-documented carcinogens (Heidelberger, 1976). Some, while not carcinogenic, may act as synergists (Hallet and Brecher, 1984; McVeety *et al.*, 1988). The United States Environmental Protection Agency (USEPA) has included 16 unsubstituted PAH in their list of 129 priority pollutants (ATSDR, 1995); these are shown in Figure 1.





Benzo[b]fluoranthene

Benzo[k]fluoranthene

Indeno[1,2,3-cd]pyrene

Benzo[a]pyrene

Benzo[g,h,i]perylene

Dibenz[a,h]anthracene

Figure 1: Showing structures of the sixteen PAH listed by United States Environmental Protection Agency as priority contaminants

The fact that chemicals could cause cancer arose from the observation of Percival Pott of St Bartholomew's Hospital, London in 1775, when he noted high incidence of cancer of scrotum among chimney sweepers who often had to climb up inside chimneys to sweep the soot down (Pott, 1777). Although he deduced correctly that the soot was responsible for the cancer, at this time, it was not possible to determine the compounds responsible for that serious tissue damage. Japanese workers later discovered that painting extracts of soot onto the skin of mice caused tumours of the skin (WHO, 1998). It was 1929 when the first pure chemical carcinogen DahA (dibenzo[a,h]anthracene) was isolated from soot extract at the Chester Beauty Research Institute by Kennaway (WHO, 1998). Doll, on the basis of wide epidemiological and statistical analysis proved in 1953 that cigarette smoking was a prime cause of lung cancer (Hall-Robets *et al.*, 2000). Careful analysis of the smoke and tar obtained from cigarettes showed that it contained many carcinogenic PAH, from which BaP (benzo[a]pyrene) was assessed as the most dangerous compound (Hall-Roberts *et al.*, 2000).

The low aqueous solubility, limited volatility and recalcitrance towards degradation allow PAH to accumulate to levels at which they may exert toxic effects upon the environment (Neff, 1985; Onuska, 1989). There is increasing concern about the contamination of many environmental substances with PAH, especially air, water and foods making human beings and aquatic organisms particularly susceptible to these pollutants. There is therefore a need for a better understanding of their formation, behaviour and fate in the environment. The history of PAH distribution and exposure in the environment can be determined from the changes in their concentration in the lake sediment with depth, after allowance is made for biodegradation and disturbances to the sediment by bioturbation and water movements (Meyers, 1976).

Kisumu City is the third largest City in Kenya. It is situated on Winam Gulf of Lake Victoria. Winam Gulf is the principal port of the lLake on the Kenyan side (Ogutu-Ohwayo et al., 2002). The City has a number of potential sources of PAH including spills from open air garages and petrol stations (without oil interceptors), car wash activities, steamers, railway maitainance shed and other petroleum based industries (David and Nancy, 1995)

1.2 Statement of the problem

The level of persistence of the 16 USEPA priority pollutants PAH in the Winam Gulf is unknown. With the increase in the potential sources of PAH in the Winam Gulf, such as oil spills, use of petroleum based engines, industrial and domestic garbage incineration, a number of carcinogenic PAH could be offloading to the Gulf waters. Significant persistence of these PAH in the sediments and waters of the Gulf would exert toxic effects on biota and eventually to humans and other terrestrial organisms. The types and concentrations of these PAH in sediment and water samples of the Gulf need to be established.

1.3 Hypothesis

The inherent PAH in lubricating oil and those from catalytic petroleum combustion processes are emitted to the waters of Winam Gulf mainly from non-point sources. Being ubiquitous contaminants, PAH accumulate in the waters and sediments of the Gulf. Determination of types and levels of PAH in the sediment and water samples of the Gulf would point towards possibility of accumulation in biota and fish from the region. The type and sources of PAH in the region can be screened for by analysis using GC-FID and GC-MS techniques.

1.4 Justification of the research

A study done by German Environmental Consultants in 1985 revealed that most petrol stations, built up and open air garages in the Winam Gulf do not have functioning oil interceptors, hence discharge oil directly into sewer lines and open grounds. Further, oil spills from Kenya pipeline station, steamers, railway maintenance shed and petroleum based industries finally discharge into the Lake (Anonymous, 1985). Another study in 1996 estimated that open air garages poured 182,500 litres of used and dirty oil per year on the ground leading to a total load of oil to the Winam Gulf of the Lake of 18,250 litres per year. It was also estimated that car wash activities in Kisumu City dumped 51,200 litres of oil per year directly into the lake based on an estimated 200 cars washed per day (David and Nancy, 1995).

Oil being a well documented source of PAH (Natus, 1978; Davies et al., 1988; Mills, 1983; Williams *et al.*, 1986a; Williams *et al.*, 1986b; Lalah and Kaigwara, 2005), there is strong need to carry out studies regularly on PAH pollution levels, especially at designated Winam Gulf sites exposed to heavy use of petroleum fractions and municipal waste disposal. No such work has been documented in the Winam Gulf region leading to lack of documented information on PAH pollution for the area. Such information is vital in the management of the Lake with reference to matters of environmental protection, developmental activities and town planning. This research therefore, sought to unravel pollution status of the Gulf by the 16 USEPA priority pollutant PAH.

1.5 Objectives

The objectives of the research were;

(i) To establish the particular United States Environmental Protection Agency listed priority pollutant Polycyclic Aromatic Hydrocarbons present in samples drawn from Winam Gulf.

(ii) Quantify these Polycyclic Aromatic Hydrocarbons in water and sediment samples from the Gulf.

(iii) Determine any fluctuations of the PAH in water and sediments samples with seasonal changes and changes in distance of sampling from the shore.

(iv) To establish the relationship between the amount of PAH in water and sediment samples.

(v) To compare some individual PAH concentration levels in the sampled Gulf water with the international standards.

1.6 Scope and limitations of the research

The research was restricted to screening for and quantifying the sixteen unsubstituted PAH, listed by USEPA as potential carcinogens, since their external standard mix was available. The PAH are acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, benzo(a)pyrene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene and pyrene (Heidelberger, 1976; ATSDR, 1995).

Time limit restricted sampling to only two points of the Gulf perceived to be representative of the most susceptible regions to PAH pollution from both point and non-point sources within the Gulf. These points were Car wash area and the lake entrance of river Kisat, which discharges a lot of raw sewage and garbage into the lake (David and Nancy, 1995).

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Identity, physical and chemical properties and analytical methods of PAH

2.1.1 Identity of PAH

Polyaromatic hydrocarbons, polyaromates, polynuclear aromatic hydrocarbons, or PAH all refer to a class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. The aromatic rings are either cata-condensed (linearly or angularly) or peri-condensed. The cata-condensed PAH are either branched or non-branched systems containing only six membered rings (WHO, 1998).

2.1.2 Physical and chemical properties

Polycyclic aromatic hydrocarbons are usually crystalline solid materials having high melting points and low vapour pressures, very low water solubilities, and usually exhibiting some colour. Because of their relatively low vapour pressures, low water solubilities, and due to their aromaticity, they exhibit a strong adsorption affinity for inorganic and organic surfaces (Onuska, 1989). PAH are considered to be associated with suspended particulate matter in both the atmospheric and aquatic environment. Some physical data for the USEPA priority pollutant PAH is given in Table 1, showing that they have high molecular weight ranging from 128.19 to 0 278.36, high melting points and boiling points ranging from 81 to 278 and 128 to 496^{0}C, respectively. Their water solubility is low ranging from 0.3 to 800 µg/L.

Table 1: Showing physical data for USEPA priority pollutant PAH

PAH	**Molecular Weight**	**Melting point (^{0}C)**	**Boiling point (^{0}C)**	**Solubility (µg/l)**
Naphthalene	128.19	81	218	-
Acenaphthene	154.21	96	279	-
Fluorene	166.23	177	294	800
Phenanthrene	178.24	101	338	435
Anthracene	178.24	216	340	59
Fluoranthene	202.26	111	383	260.0
Pyrene	202.26	156	393	133.0
Benzo(a)anthracene	228.30	162	435 sub	11.0
Chrysene	228.30	256	441	1.9
Dibenz(a,h)anthracene	278.36	270	-	-
Benzo(g,h,i)perylene	276.34	278	-	0.3
Acenaphthylene	152.21	93	270	-
Benzo(b)fluoranthene	252.32	168	481	2.4
Benzo(k)fluoranthene	252.32	217	481	-
Benzo(a)pyrene	252.32	177	496	3.8
Indeno(1,2,3-cd)pyrene	276.34	-	-	-

Key: sub: "sublimes", - unavailable in literature
Source: Onuska, 1989.

Although PAH comprise a diverse range of organic compounds, some general trends in physical-chemical properties are apparent. As the molecular weight of PAH increases, their vapour pressures enhance saturation vapour hence solubility decreases. At elevated temperatures, some PAH, especially the lower molecular weight compounds, exist particularly in the vapour phase. Loss of material due to volatilization during ambient atmospheric sampling over relatively long periods has been reported (Lee *et al.*, 1981). The published values of the solubilities of PAH indicate a wide variation, reflecting in part the differences in preparation and determination of PAH solutions. In general, the trends observed can be summarized as:

- Water solubility of PAH decreases, as their molecular weight increases.
- lineary fused aromatic rings, such as naphthalene and anthracene, are usually less soluble than angular or peri-condensed PAH such as chrysene and phenanthrene.
- Alkyl substitution of the aromatic ring decreases water solubility (WHO, 1998).

Temperature has a significant influence on the solubility of PAH, as can be demonstrated in the case of water solubility of anthracene. Its solubility increases from 12.7 ± 0.5 μg/L to 55.7 ± 0.7 μg/L when the temperature is increased from 5 to 29°C (WHO, 1998). PAH undergo chemical reactions characteristic of organic aromatic molecules, including 1,2-additions, 1,4- additions and electrophilic substitutions (Onuska, 1989). The type of reaction that occurs, as well as the rate of reaction, is dependent upon the molecular structure of the particular PAH in addition to the reagent and the reaction medium.

In the presence of ultra violet light and oxygen (oxic conditions), PAH readily undergo photo-oxidation (Onuska, 1989). Different PAH absorb radiation at various wavelengths. Absorption of incident radiation results in activation of the ground state molecule to an excited singlet state which may react with a second ground state molecule to produce a photo product or undergo a transition to an excited triplet state (Onuska, 1989). Reaction of the excited triplet state molecule with ground state molecular oxygen produces excited singlet state PAH molecule which yield oxygenated products. PAH can undergo further dealkylation and ring cleavage during photolysis. One-electron oxidations can also occur, producing radical cations. Being unstable, radical cations react with water molecules or other nucleophiles to produce diols, quinones, aldehydes, and dimmers (Onuska, 1989). PAH are soluble in very many organic solvents and are highly lipophilic. At atmospheric conditions, they are chemically rather inert. The only reactions that are of interest with respect to their environmental fate and possible sources of loss during atmospheric sampling are photodecomposition and reactions with nitrogen oxides, nitric acid, sulphur oxides, sulphuric acid, ozone, and hydroxyl radicals (Onuska, 1989).

2.2 Sources of PAH in the environment

Little information is available on the production and formation of PAH, but it is probable that only small amounts of PAH are released as a direct result of production and formation activities (NAS, 1971). The PAH formed are principally used as intermediates in the production of polyvinylchloride and plasticizers (naphthalene), pigments (acenaphthene, pyrene), dyes (anthracene, fluoranthene), and pesticides (phenanthrene). PAH are generally not produced commercially in the United States except as research chemicals. However, PAH are found in coal, coal tar, and in the creosote oils, oil mists, and pitches formed from the distillation of coal tars (ATSDR, 1995).

The largest emissions of PAH result from incomplete combustion of organic materials during industrial processes and from other human activities including:

- Processing of coal, crude oil, and natural gas, including coal coking, coal conversion, petroleum refining and production of carbon blacks, creosote, coal-tar, and bitumen;
- Aluminum, iron and steel production in plants and foundries;
- Heating in power plants and residences;
- Combustion of refuse;
- Motor vehicle exhaust emissions; and
- Environmental tobacco smoke (Longwell, 1983; WHO, 1998; Weilmuenster *et al.*, 1999).

PAH, especially those of higher molecular mass enter the environment, via the atmosphere, adsorbed onto particulate matter (WHO, 1998). The hydrosphere and geosphere are affected secondarily by wet and dry deposition. Creosote-preserved wood is another source of release of PAH into the hydrosphere, and deposition of contaminated refuse, like sewage sludge and fly ash, contributes to emissions of PAH into the geosphere. PAH occur naturally in peat, lignite, coal and crude oil. Most of the PAH in hard coal are tightly bound within the coal structure and cannot be leached out (WHO, 1998).

As a result of incomplete combustion, it is believed that a diesel engine emits over 10,000 compounds either in gas phase or adsorbed onto carbon particles given off in form of soot (Lipkea, 1978). Diesel powered vehicles emit, on a total mass basis, approximately 30 to 100 times more particulate matter than gasoline powered internal combustion engines (Lipkea, 1978; Anonymous, 1983). It is suggested that the formation of these particulates and their chemical nature are influenced by chemical compositions of the fuel used as well as the combustion characteristics in the engine (Lipkea, 1978; Andrew *et al.*, 1983; Olten and Senkan, 1999; Schulz *et al.*, 1999; Lalah and Kaigwara, 2005). The nature of these particulates varies depending on the chemical composition of the fuel, i.e. its C, H, Cl, S and trace metal content and the degree of aromaticity of the fuel. The particulates are defined here as any dispersed matter collected on filter papers at a temperature below 52^0C excluding condensed water (Barth and Blacker, 1978). Studies have shown that these particulates contain PAHs (Andrews *et al.*, 1983; Schulz and Sinks, 2002, Lalah and Kaigwara, 2005). These particulates are emitted into the atmosphere and eventually can find their way back into soil and into aquatic environment.

The origin of PAH in exhaust emissions has been an important subject of research addressing the question as to whether they are formed during the combustion process or are simply unburnt fuel or a combination of both (Wakeham *et al.*, 1980 b; Sporstal *et al.*, 1983; Budzinski *et al.*, 1997). From analysis of high boiling-point residues obtained from catalytic reactors of refineries, it was found that large amounts of PAH were formed in the catalytic cracking processes during refining (Lipkea, 1978).

The PAH are inherent in fuel and in lubricating oils and these therefore can contribute to the type and amount of emitted PAH (as unburnt hydrocarbons) from the engine exhaust (Mills, 1983; Williams *et al.*, 1986 a; Williams *et al.*, 1986 b; Davies *et al.*, 1988). Experimental evidence deduced from recent combustion studies done on a variety of fuels indicates that chemical kinetics during combustion is the dominant formation process that influences exhaust particulate emissions and that PAH are also combustion generated (Weilmuenster *et al.*, 1999; Hall-Roberts *et al.*, 2000). The composition of diesel fuels is extremely complex consisting of hydrocarbons, oxygenated organic compounds, parent and substituted PAH, nitrogen, sulphur, heterocyclic and inorganic compounds (Anonymous, 1985).

Domestic and residential heating emit phenanthrene, fluoranthene, pyrene and chrysene as major PAH components (WHO, 1998). The emissions from wood stoves is 25-1000 times higher than those from charcoal-fired stoves, and in areas where wood burning predominates for domestic heating, the major portion of airborne PAH may be from this source. The main compounds released from petrol-fuelled vehicles are fluoranthene and pyrene while naphthalene and acenaphthene are abundant in the exhaust of diesel-fuelled vehicles. The exhaust emission rates depend on engine conditions and may range from a few nanograms per kilometer to more than 1000 mg/km (WHO, 1998). PAH emissions from vehicle engines are dramatically reduced by fitting catalytic converter devices (WHO, 1998).

2.3 Environmental distribution

Every year, 230 000 tonnes of PAH reach the marine environment and are ubiquitously distributed world-wide (Law, 1986). Several distribution and transformation processes determine the fate of both individual PAH and their mixtures. They travel through the atmosphere as a gas or attached to dust particles. They are carried by air currents and deposited by dry or wet (rain, dew) deposition. When deposited in water, they sink to the bottom of lakes and rivers. Some move through the soil and contaminate ground water (Christensen *et al.*, 1997; WHO, 1998).

Partitioning between water and air, between water and sediment and between water and biota are the most important of the distribution processes. As PAH are hydrophobic with low solubilities in water, their affinity for the aquatic phase is very low, however, in spite of the fact that most PAH are released into the environment via the atmosphere, considerable concentrations are also found in the hydrosphere (WHO, 1998).

As the affinity of PAH for organic phases is greater than that for water, their partition coefficients between organic solvents, such as octanol, relative to water are high. Their affinity for organic fractions in sediment, soil, and biota is also high, and PAH thus accumulate in organisms in water and sediments and in their food chain. Benzo(a)pyrene bioaccumulates in sediment and biota at 100 to 10,000 times the level found in water. PAH biodegradation in microorganisms and metabolism in higher biota are important pathways in the environment since more carcinogenic metabolites may be formed (WHO, 1998).

In the Baltic Sea, very low concentrations ranging from 0.001 – 4.8 ng/L of PAH have been reported outside coastal areas during autumn (Witt, 1995). Two and three ring aromatics like naphthalene, acenaphthene and phenanthrene were found to predominate, whereas the concentration of the higher-molecular-weight five and six ring compounds remained below 0.1 ng/L.

The concentration of PAH in spring has been found to be below that measured during autumn with their lowest concentration being measured in summer (Witt, 1995). This concentration variation has been attributed to seasonal changes in degradation intensity (Readman *et al*., 1984; Whitehouse, 1984). During the winter period, low sea water temperatures limit the microbial decomposition of PAH. The photo-oxidation of these compounds, which is correlated to the light intensity, is also lower in autumn. On the other hand, during the spring plankton bloom, a high amount of detrital material is produced that might effectively scavenge PAH and transfer them to the sea floor. Another possible explanation could be the increased atmospheric input of PAH in autumn (Jensen, 1983).

The seasonal distribution patterns are more significant for the low-molecular-weight PAH (two to three rings) due to their higher sensitivity to photo-oxidation and microbial degradation, whereas the four to six ring PAH do not show any significant differences due to their high persistence for microbial degradation (Witt, 1995).

The concentration of PAH measured in the surface water micro-layer has been found to be 2-13 times higher than in the water below (Witt, 1995). The enrichment of PAH in the micro-layer results mainly from the atmospheric input. However, the concentrations of PAH are higher in sea water near the sea floor than in the water just below the subsurface (Witt, 1995). The elevated concentrations in the bottom water has been attributed to the contribution of resuspended sediment particles.

In coastal marine environments, river run-off is an important source of PAH contamination (Herrmann and Hubner, 1982). This implies that the concentrations of PAH in rivers and estuaries should be higher than in the open sea, consequently, concentration of the aromatics decrease with increasing distance from the river mouth (Witt, 1995).

The majority of PAH in sediments are thought to be derived from the combustion of fossil fuels (Hites *et al.*, 1977). Due to their lipophilic character and their high persistence, PAH can accumulate in the sediments (Neff, 1979). PAH persistence increases with increasing molecular weight (Witt, 1995). PAH in the sediments are mainly associated with the organic matter (Evans *et al.*, 1990). High amounts of PAH occur in sediments with a high total organic carbon.

In the sediments, the higher- molecular-weight and more lipophilic and hydrophobic PAH predominate probably due to their higher persistence (Witt, 1995). The higher-molecular-weight aromatics are preferentially adsorbed by particulate matter and incorporated in the sediment (Witt, 1995).

2.3.1 Air

In temperate regions, the level of individual PAH tend to be higher in winter than in summer by at least one order of magnitude. The predominant source during winter is residential heating, while that during summer is urban motor vehicle traffic emissions. Average concentrations of 1-30 ng/m^3 of individual PAH have been detected in the ambient air of various urban areas (WHO, 1998). In large cities with heavy motor vehicle traffic and extensive use of biomass fuel, levels of up to 200 ng/m^3 have been detected (WHO, 1998). These allegations are yet to be ruled out in developing countries such as Kenya where more than 70% of energy consumption is based on biomass combustion.

2.3.2 Surface water and precipitation

Most of the PAH in water are believed to result from urban run off, from atmospheric fallout (smaller particles), and from asphalt abrasion (larger particles) (WHO, 1998). The major sources of PAH vary, however, in a given body of water. In general, most samples of surface water contain individual PAH at levels of up to 50ng/litre but highly polluted rivers could have up to 6 000 ng/litre (WHO, 1998).

2.3.3 Sediment

The concentrations of individual PAH in sediment are generally one order of magnitude higher than those in precipitation (WHO, 1998). Hydrocarbons, including PAH, in sediments can have several sources, as is the case for hydrocarbons in the marine environment in general. These can be grouped into the following categories:

- Petroleum inputs into the ocean as shown in Table 2.
- Hydrocarbons, especially PAH released as a result of partial combustion of fuels (such as gas, coal, oil and wood) for transportation, heating and power generation.
- PAH originating in forest and grass fires, transported to the marine environment through aeolean and fluvial processes. This source is usually important to consider only at the ppm (µg/g dry weight) or less concentration level.
- Biosynthesis of selected (biogenic) hydrocarbons by marine or terrigeneous organisms.
- Early diagenesis transformations in sediments or in the overlying water column of non-hydrocarbon biosynthetic natural products to hydrocarbons. An example is the microbial and possibly chemical transformation of sterols and hopanols to partially aromatized hydrocarbons (Brassel *et al.*, 1981). This source is quantitatively important in organic rich sediments and in areas receiving sewage and sewage sludge inputs and when analyzing for pollutant hydrocarbons at the ppm concentration levels or less (UNEP, 1992).

Table 2: Showing input of petroleum hydrocarbons, including PAH, into the marine environment (million metric tonnes per annum).

Source	probable range.
Natural sources	
Marine seeps	0.02 – 2.0
Sediment erosion	0.005 – 0.5
Offshore production	0.04 – 0.06
Transportation	
Tanker operations	0.4 – 1.5
Dry docking	0.02 – 0.05
Marine terminals	0.01 – 0.03
Bilge and fuel oils	0.2 – 0.6
Tanker accidents	0.3 – 0.4
Non-tanker accidents	0.02 – 0.04
Atmosphere	0.05 – 0.5
Municipal and industrial runoff	
Municipal wastes	0.4 – 1.5
Refineries	0.06 – 0.6
Non-refining industrial waste	0.1 – 0.3
Urban runoff	0.01 – 0.5
River runoff	0.01 – 0.5
Ocean dumping	0.005 – 0.02

Source: NAS, 1985

Sediments play an important role in uptake of PAH by some species since PAH dissolved in sea water are accumulated from sediment (WHO, 1998). The ratio of the concentration of an individual PAH in a bottom dwelling organism and in the sediment is such that the bioaccumulation factor is usually < 1 when expressed as wet weight. It is therefore important that the concentration of PAH in sediments is regularly monitored in the environment (WHO, 1998).Table 2 indicates that oil spills, municipal wastes and atmospheric deposition are very significant sources of PAH input into large aquatic environments. It is therefore expected that Winam Gulf, at Car Wash and Kisat river entrance, would indicate significant levels of PAH in sediment and water.

2.3.4 Aquatic organisms

Marine organisms are known to absorb PAH from water and accumulate them in their bodies. The degree of contamination in water and sediments is related to the extent of industrial and urban development, and shipping movements. PAH concentrations of up to 7 mg/kg have been detected in aquatic organisms living near industrial effluents (WHO, 1998).

The average levels of PAH in aquatic animals sampled at PAH contaminated sites have been found to be 10–500 μg/kg, although levels of up to 5 mg/kg have also been detected (WHO, 1998). The average levels of PAH in aquatic animals sampled at various sites with unspecified sources of PAH have been found to be 1-100 μg/kg, but concentrations of up to 1 mg/kg have been detected in lobsters in Canada (WHO, 1998).

2.3.5 General human population

The main sources of non-occupational exposure are: polluted ambient air, smoke from open fire places and cooking, environmental tobacco smoke, contaminated food and drinking-water, and the use of PAH-contaminated products. PAH can be found in indoor air as a result of residential heating and environmental tobacco smoke at average concentrations of 1-100 ng/m^3, with a maximum of 2300 ng/m^3 (WHO, 1998).

The intake of individual PAH from food has been estimated to be 0.10-10 μg per person. The total intake of benzo(a)pyrene from drinking water has been estimated to be 0.0002 μg/person (WHO, 1998). Cereals and cereal products are the main contributors to the intake of PAH from food because they are a major component of the total diet (WHO, 1998).

2.4 Kinetics and metabolism

Absorption of PAH into the human body is mainly through the pulmonary tract, the gastrointestinal tract, and the skin. The rate of absorption through the lungs depends on the type of PAH, the size of the particles on which they are adsorbed, and the composition of the adsorbent (WHO, 1998). PAH adsorbed onto particulate matter are cleared from the lungs more slowly than free hydrocarbons. Absorption from the gastrointestinal tract occurs rapidly in rodents, but metabolites return to the intestine via biliary excretion (WHO, 1998). Studies done with ^{32}P-post labeling of percutaneous absorption of PAH mixtures in rodents showed that components of the mixtures reach the lungs, where they become bound to DNA (WHO, 1998).

There is wide distribution of PAH throughout the organism after administration by any route and they are found in almost all internal organs, but particularly those rich in lipids. Intravenously injected PAH are cleared rapidly from the blood stream of rodents but can cross the placental barrier and have been detected in fetal tissues. The metabolism of PAH is complex, but in general, parent compounds are converted via intermediate epoxides to phenols, diols and tetrols, which can themselves be conjugated with glucuronic acids or with glutathione. Most metabolism results in detoxification, but some PAH are activated to DNA-binding species, principally diol epoxides, which can initiate tumours (WHO, 1998).

The metabolites of PAH and their conjugates are excreted via the urine and faeces, but conjugates excreted in the bile can be hydrolyzed by enzymes of the gut and reabsorbed (WHO, 1998). PAH do not persist in the body and have rapid turnover, except those PAH moieties that become covalently bound to tissue constituents. Benzo(a)pyrene, when administered by the oral route, produced tumours of the gastrointestinal tract (forestomach), liver, lung and mammary glands of mice and rats (WHO, 1998).

2.5 Effects on humans

If you are exposed to substances such as PAH, many factors will determine whether harmful health effects will occur and what the type and severity of those health effects will be. These factors include the dose (how much), the duration (how long), the route or pathway by which you are exposed (breathing, eating, drinking, or skin contact), the other chemicals to which you are exposed, and your individual characteristics such as age, sex, nutritional status, family traits, lifestyle, and state of health (ATSDR, 1995).

After dermal application, anthracene, fluoranthene, and phenanthrene have been found to induce specific skin reactions, and benzo(a)pyrene, to induce reversible regressive verrucae which are classified as neoplastic proliferations (WHO, 1998). The typical effect of naphthalene, after dermal or oral exposure, is acute haemolytic anaemia, which can also affect fetuses transplacentally (WHO, 1998). The systematic effects of naphthalene are known from numerous cases of accidental intake, particularly by children. The lethal oral dose is 0.03 – 0.3 mg/kg body weight for adults (depending on body weight and PAH type), and 2000mg for a child, taken over two days (WHO, 1998).

Tobacco smoking is the most important single factor in the induction of lung tumours and also for increased incidences of tumours of the urinary bladder, renal pelvis, mouth, pharynx, larynx, and oesophagus (WHO, 1998). Occupational exposure to soot as a cause of scrotal cancer was noted for the first time in 1775 (WHO, 1998). Later, occupational exposure to tars and paraffins was reported to induce skin cancer. The lung is now the main site of PAH – induced cancer, whereas skin tumours have become more rare because of better personal hygiene. Increased lung tumour rates due to exposure to PAH have been found in coke-oven workers and asphalt workers (WHO, 1998).

The risk of the general population developing lung cancer over a lifetime has been calculated to be 10.4 to 10.5 per ng of benzo(a)pyrene per m^3 air, translating to about one person in 10,000 or 100,000 developing lung cancer in his or her lifetime as a result of exposure to benzo(a)pyrene in air (ATSDR, 1995). The genotoxic effects of PAH have been determined by testing for mutagenicity in urine and faeces and for the presence of micronuclei, chromosomal aberrations and sister chromatid exchange in peripheral blood lymphocytes (ATSDR, 1995).

The American government has set regulations to protect people from the possible health effects of eating, drinking, or breathing PAH. Environmental Protection Agency (EPA) has suggested that taking into your body each day the following amounts of individual PAH is not likely to cause any harmful health effects: 0.3 milligrams (mg) of anthracene, 0.06 mg of acenaphthene, 0.04 mg of fluoranthene, 0.04 mg of fluorene, and 0.03 mg of pyrene per kilogram (kg) of your body weight (one kilogram is equal to 2.2 pounds) (ATSDR, 1995). The American government has developed regulatory standards and guidelines to protect people from the potential health effects of PAH in drinking water. EPA has provided estimates of levels of total cancer-causing PAH in lakes and streams associated with a risk of human cancer development. The following are the EPA lower pollution limits of individual PAH in the environment within a 24-hour period: benzo[b]fluoranthene, benzo[a]pyrene, or dibenz[a,h]anthracene, (0.454 kg); benz[a]anthracene, (4.54 kg); acenaphthene, chrysene, fluoranthene, or indeno[1,2,3-c,d]pyrene, (45.4 kg); or acenaphthylene, anthracene, benzo[k]fluoranthene, benzo[g,h,i]perylene, fluorene, phenanthrene, or pyrene, (2267.96 kg) (ATSDR, 1995; WHO, 1998).

In the United States of America, National Institute for Occupational Safety and Health (NIOSH) concluded that occupational exposure to coal products can increase the risk of lung and skin cancer in workers. It established a recommended occupational exposure limit, time-weighted average (REL-TWA) for coal tar products of 0.1 milligram of PAH per cubic meter of air (0.1 mg/m^3) for a 10-hour workday, within a 40-hour workweek (ATSDR, 1995). The American Conference of Governmental Industrial Hygienists (ACGIH) recommends an occupational exposure limit for coal tar products of 0.2 mg/m^3 for an 8-hour workday, within a 40-hour workweek. The Occupational Safety and Health Administration (OSHA) for United states of America lobour department has established a legally enforceable limit of 0.2 mg/m^3 averaged over an 8-hour exposure period. The OSHA Permissible Exposure Limit (PEL) for mineral oil mist is 5 mg/m^3 averaged over an 8-hour exposure period. NIOSH has concurred with this limit, and has established REL-TWA for mineral oil mists of 5 mg/m^3 for a 10-hour work day, 40-hour work week, with a 10 mg/m^3 Short Term Exposure Limit (STEL) (ATSDR, 1995).

The United States of America, Department of Health and Human Services (DHHS) has determined that some PAH are reasonably carcinogenic (ATSDR, 1995). In the body, PAH are changed into chemicals that can attach within the body and special tests can detect these chemicals in body tissues or blood. However the tests cannot tell whether any health effects will occur or find out the extent or source of exposure. Table 3 below shows results of tests for genotoxicity and carcinogenicity of the 16 USEPA priority pollutant PAH (WHO, 1998).

Table 3. Showing results of genotoxicity and carcinogenicity tests for the 16 USEPA Priority pollutant PAH

Compound	Genotoxicity	Carcinogenicity
Acenaphthene	(?)	(?)
Acenaphthylene	(?)	No studies
Anthracene	–	–
Benzo(a)anthracene	+	+
Benzo(b)fluoranthene	+	+
Benzo(k)fluoranthene	+	+
Benzo(ghi)perylene	+	–
Benzo(a)pyrene	+	+
Chrysene	+	+
Dibenz(a,h)anthracene	+	+
Fluoranthene	+	(+)
Fluorene	–	–
Indeno(1,2,3-cd)pyrene	+	+
Naphthalene	–	(?)
Phenanthrene	(?)	(?)
Pyrene	(?)	(?)

Key:
+, positive ?, questionable
_, negative ( ), results derived from small database.

Source: WHO, 1998

2.6 Methods of Study

2.6.1 Sampling

Sediment sampling has been done using grab samplers for sandy sediments but a bottom corer is preferred in getting sediments under water since it does not lose surface layers as it is brought up through the water column (Riddle, 1989). The initial arrival of PAHs to the sediment may be in the form of fine flocculent material at the sediment / water interface, a layer which is not retained by most grab and coring devices (Baxter *et al*, 1981).

Water sampling is done at subsurface (just below surface), near surface (1 m below surface) or near bottom (1 m from the bottom) with samplers which must be deployed and retrieved in the closed position (Boehn, 1995). The sample jars are lowered by hand and opened or un-capped only at the sampling depth. The water samples are immediately placed in cooler and kept at 4^0C, but should not be frozen (Boehn, 1995). Water samples can be held at 4^0C in the dark for up to 7 days without loss of sample integrity, while water extracts can be held for 40 days under the same conditions without loss of sample integrity (Boehn, 1995)

2.6.2 Sample extraction

Many extraction and purification techniques depend on the matrix. PAH are extracted from samples using Soxhlet apparatus, ultrasonically, by liquid-liquid partition after sample dissolution or alkaline digestion, with a selective solvent (Novotny *et al*., 1984).

Solvent extraction is attractive when the sediment mainly consists of clay particles polluted with contaminants which are not easily biodegradable (Novotny et al., 19884)

Extracted samples are usually purified by column chromatography, particularly on alumina, silica gel, or sephadex LH-20 and also by thin layer chromatography. Silica gel has been used most frequently where aliphatic hydrocarbons are eluted with n-pentane and aromatic hydrocarbons with benzene (Novotny *et al.*, 1984; Williams *et al.*, 1986a). Silica gel and alumina, used in the routine clean up process, have been shown to catalyze photo-oxidation of PAHs (Lee *et al.*, 1981), resulting in decreased recoveries.

2.6.3 Identification and quantification by Gas Chromatography and High Pressure Liquid Chromatography

Identification and quantification are routinely performed by gas chromatograph with flame ionization detection (GC-FID) or by high performance liquid chromatography (HPLC) with ultra violet or fluorescence detection. In gas chromatography, fused silica capillary columns are used, with polysiloxanes (SE-54 and SE-52) as stationary phases; silica-C18 columns are commonly used in HPLC. GC-MS analysis has been reported to provide low detection limits and good reproducibility (Wise *et al.*, 1993), while GC-FID has been shown to be suitable for sites with high level contaminants (Christopher *et al.*, 1995). GC-MS is often used to confirm the identity of peaks (Andrews *et al.*, 1983; Anonymous, 1983).

In quantification, the chromatograph is calibrated by injecting an appropriate standard mixture and the surrogate standard used in the analysis. Response factors (RFs) are calculated in terms of peak area.ng^{-1}. This is done for the peaks on the basis of area units calculated by an electronic integrator. With vaporizing injectors, the high boiling compounds are not eluted as efficiently as the lower boiling compounds and so the response factors generally decline from C26 through C36. Hydrocarbons in samples are determined by injecting a known aliquot of the fractionated extracts into the GC (UNEP, 1992). Peak areas are electronically integrated and used to calculate the concentrations as in equation 1. The areas of the surrogate standards are used to compute the recovery through the analytical procedure.

$$(\text{ng.g}^{-1}\text{ dry weight}) = \frac{Peak\ area\ on\ GC \times RF \times XF}{g\ dry\ weight} \ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

RFs from external standard run are computed and tabulated as peak area.ng^{-1}.

XF (dilution factor) is computed as total extract volume (μL) divided by μL injected.

If necessary this result can be corrected for the recovery of the surrogate standard through the procedures. Individual compounds are reported as ng.g^{-1} dry weight (UNEP, 1992).

2.6.4 External Standard Calibration

It involves comparison of instrument's response from the sample to the responses from target compounds in the calibration standards. Sample peak areas (or heights) are compared to peak areas (or heights) of standards. This is achieved by use of calibration factor (CF) or least squares regression (LSR). The CF is the ratio of detector response to the amount (mass) of analyte in the calibration standard and is calculated as shown in equation 2. (UNEP, 1992).

$$\text{CF} = \frac{Peak\ area\ (or\ height)\ of\ compound\ in\ std}{Mass\ (concentration)\ of\ compound\ in\ std} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(2)$$

In least square regression, a linear regression of instrument response vs the concentration of a standard is obtained with instrument response as the dependent variable (y) and concentration as the independent variable (x).

The regression produces a slope and intercept in terms of a linear equation of the form shown in equation 3.

$$y = ax + b \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(3)$$

Where: - y = Instrument response (peak area or height).

a = Slope of the line (also called coefficient of x).

x = Concentration of the calibration standard.

b = the intercept

The regression calculation generates a correlation coefficient (r), which is a measure of "goodness of fit`` of the regression line to the data. In order to be used for quantitative purposes, (r) must be greater or equal to 0.99 (UNEP, 1992). The sample concentrations from this model are obtained by making x in equation 3 the subject of the regression equation to obtain equation 4..

$$x = \frac{y - b}{a} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(4)$$

2.6.5 Internal Standard Calibration

This involves comparison of instrument responses from the target compounds in the sample to the responses of specific standards added to the sample or sample extract prior to injection. The response factor (RF) which is the ratio of the peak area (or height) of the target analyte in the sample or sample extract to the peak area (height) of the internal standard in the sample or sample extract (equation 5), is compared to a similar ratio derived for each calibration standard. The minimum acceptable response factor in quantitative analysis is approximately 0.01 for the least responsive compound (UNEP, 1992).

$$RF = \frac{As \times Cis}{Ais \times Cs} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

Where:-

As - peak area (or height) of the analyte.

Ais - peak area (or height) of internal standard.

Cs - concentration of the analyte.

Cis - concentration of the internal standard.

However internal standard calibration is not as useful for GC and HPLC methods with non-MS detectors because of the inability to chromatographically resolve many internal standards from the target compounds (UNEP, 1992).

2.7 Study area

Lake Victoria is situated at $0^{0}21'$N-$3^{0}0'$S, $31^{0}39'$-$34^{0}53'$E astride the equator on an altitude of 1240 m above sea level with a surface area of 68800 sq. km (Ogutu-Ohwayo *et al.*, 2002). Kisumu City is situated at 0^{0}6’S, 34^{0}45’N (Wikipedia, 2006) on the North tip of the Winam Gulf, which is part of the Kavirondo Gulf of the Lake Victoria. It is the third largest city in Kenya as well as the principal port of the lake on the Kenyan side. It has a population of 322,724 according to 1999 population census.

In the recent past, Jua-kali sector (small holder industries) has received great attention from the government and donor countries and has rapidly expanded. By 1996, the registered Kisumu Jua-kali association had a membership of 10000 operating about 10000 working spaces in the town with the major activity of repairing diesel injectors, major vehicle overhaul, panel beating and spraying of motor vehicles (David and Nancy, 1995). Next to these garages were small-scale mechanics dealing with minor vehicle mechanical problems. These garages contribute to an estimated total load of used oil to the lake of 182500 litres oil per year (David and Nancy, 1995), making the pollution situation described above more serious. Washing of vehicles on the pavements of streets and in the lake, which started in 1960, has also recently increased as a commercial activity, discharging oil spills directly into the open Gulf waters. Railway wagons and tankers transporting oil to the depots are cleaned on site and the railway harbour, which started operating in 1901, occasionally causes localized oil spills from the fuelling points. The oil and diesel spills after servicing of the trains and ships contribute to localized pollution around the Kisumu bay. The fuel storage tanks are washed once every 5-10 years and the resultant water dumped into drains. Car washing, including engine wash, spill approximately 51200 litres of oil per year for an average 200 cars per day (David and Nancy, 1995). Significant PAH pollution is therefore expected in this area, especially near the Car Wash where the above activities are concentrated.

This study was undertaken to analyse sediment and water samples drawn from Winam gulf of Lake Victoria in Kenya. Car Wash area of the Gulf and the entrance of river Kisat into the Gulf were selected to represent the Gulf because they are highly susceptible to PAHs pollution from point and non-point sources, mainly from Kisumu City, situated in the Kisumu Bay of Winam Gulf (Figures 2). This analysis was meant to bridge the knowledge gap created by lack of documented information on the types and quantities of PAH in the Gulf.

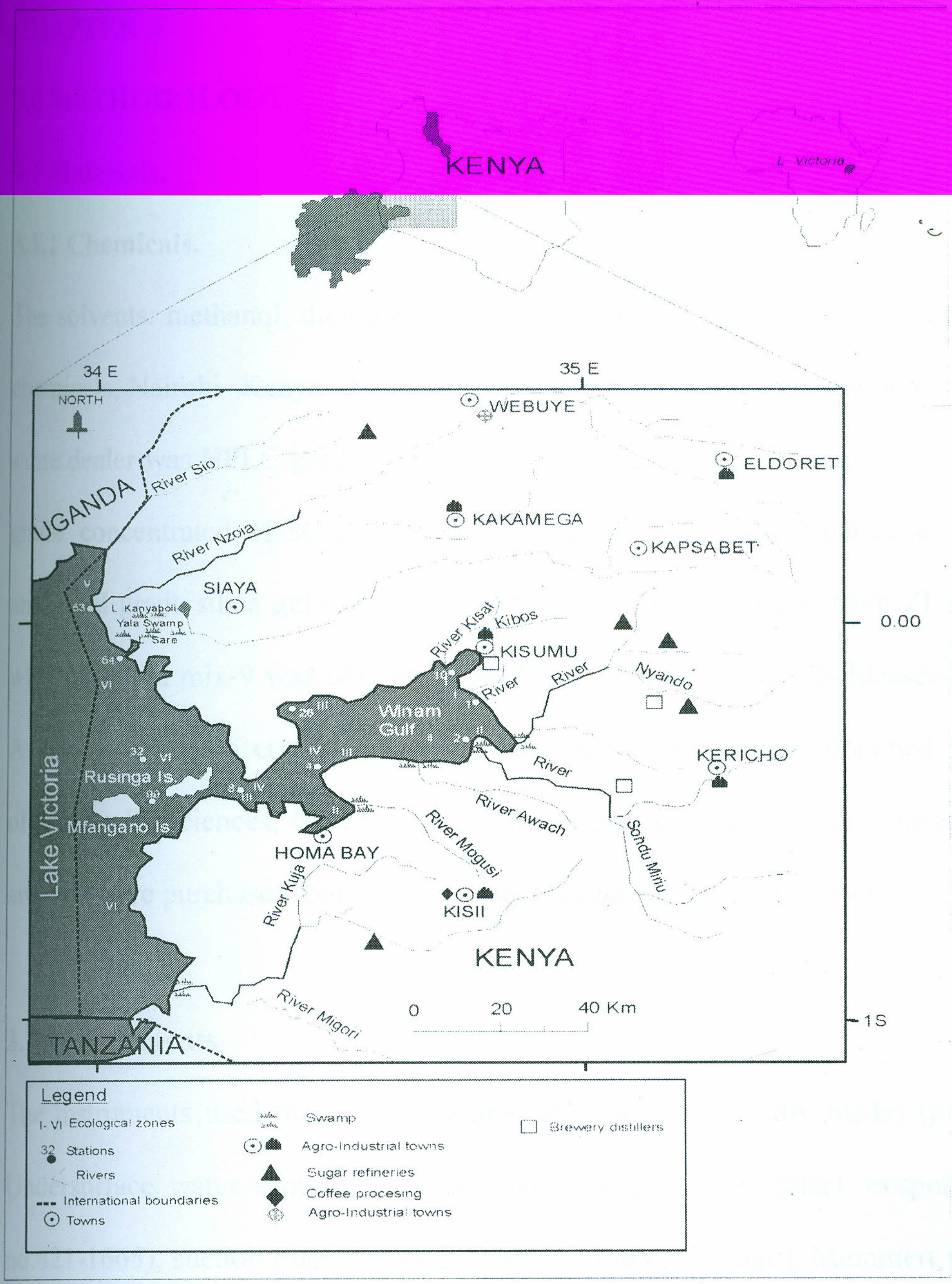


Figure 2: Map of Winam Gulf showing the location of Kisumu City and surrounding features

Source: School of Environment and Earth Sciences, Maseno University, Kenya.

CHAPTER 3

3.0 METHODOLOGY

3.1 Materials.

3.1.1 Chemicals.

The solvents: methanol, dichloromethane and toluene used were purchased from ZETA chemicals, Nairobi- Kenya and were all GPR grades. The n-Hexane purchased from the same dealer was HPLC grade, 95% pure. Analytical grade anhydrous Na_2SO_4, analytical grade concentrated H_2SO_4, analytical grade 2,4-dinitophenylhydrazine (DNPH) and analytical grade silica gel (230 - 400 mesh) were also purchased from ZETA chemicals while standard mix-9 was obtained from Dr. Ehrenstorfer, Bgm-Schlesser-Str 6A.86199 Augsburg Germany, certified sediment and standard water were obtained from Institute of Marine Biosciences, Canada. Pure nitrogen, hydrogen and oxygen gases for GC-FID analysis were purchased from East African Oxygen Company in Kisumu.

3.1.2 Instruments

The instruments used included: Sediment grab sampler (locally made) (plate 1 and 2), Undersurface water sampler (locally made), deep freezer, rotary evaporator (model no.421-1665), suction pump (model 7049-05, 230V, Chicago), Memmert Oven, Orbital shaker (SO1 Stuart Scientific model), analytical balance (Fisher Scientific A-16, USA) and Gas Chromatograph (GC-17A Ver.3 SHIMADZU).

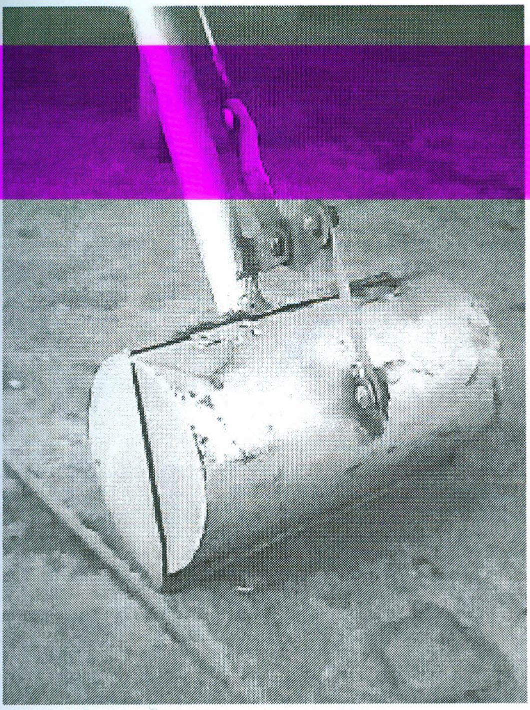


Plate 1: Locally made grab sampler
closed position

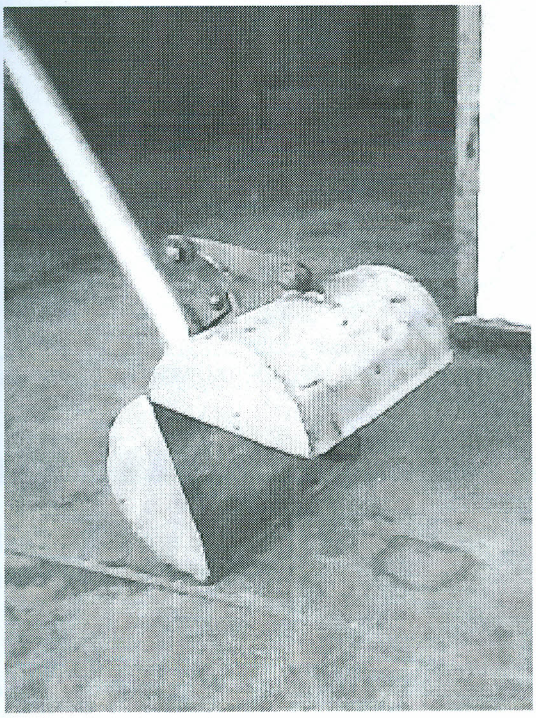


Plate 2: Locally made grab sampler
Open position

3.2 Analytical methods

3.2.1 Sampling and sample preparation

Water and sediment sampling was done from the two sampling sites, Kisat (1X) and Car Wash (2X) (Figure 3)

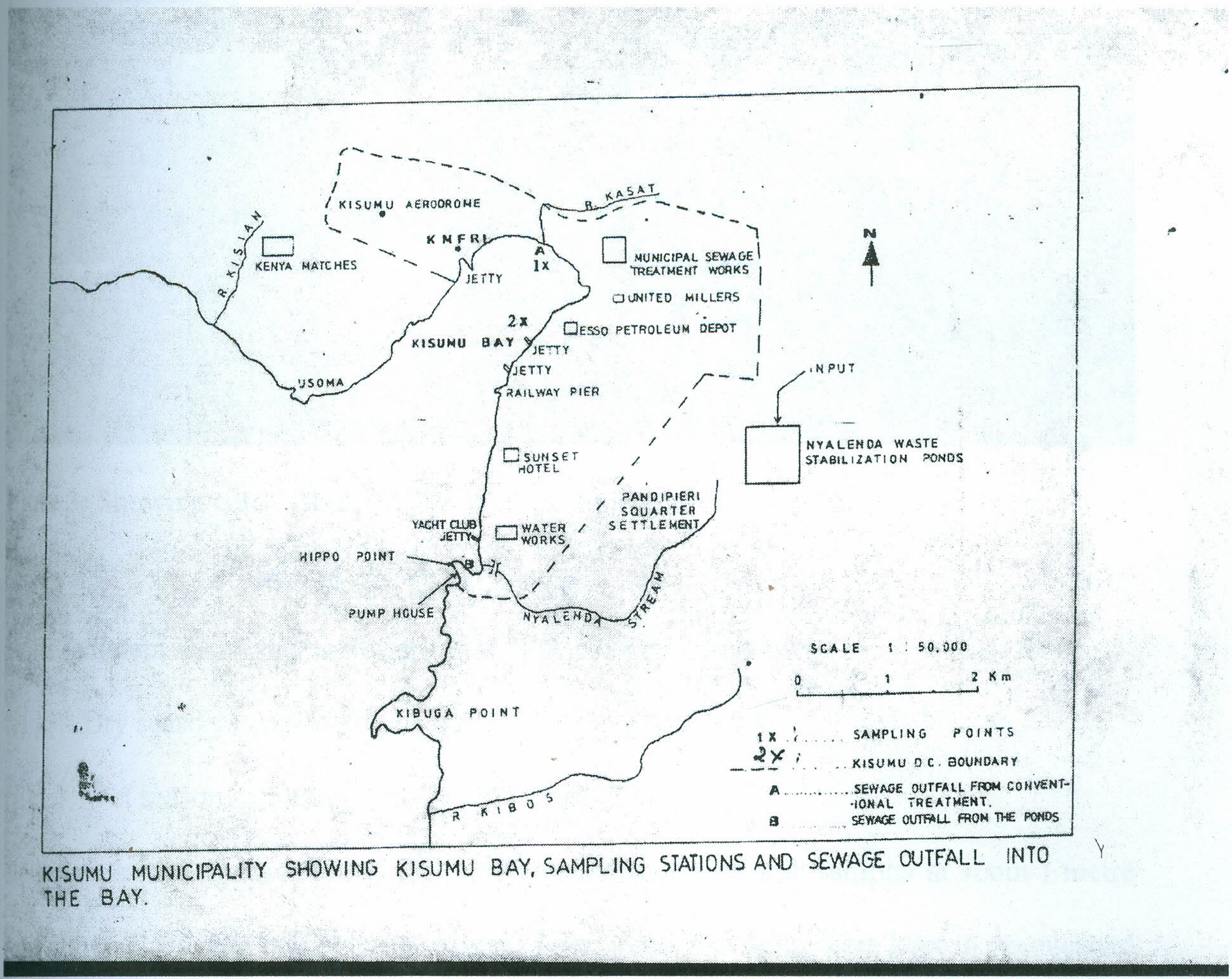


Figure 3: Map of Kisumu Bay showing the sampling sites
Source: Kisumu Municipality Town Hall

During sampling at Car Wash, between 30 to 50 cars were found being washed (Plate 3) and this was a probable anthropogenic source of PAH in this area.

Plate 3: Showing car washing at Car Wash sampling site

Both sediment and water sampling were done over two seasons:

(i) Dry season sampling done on 28th October, 2005

(ii) Wet season sampling done on 20th April, 2006.

Sediment sampling was done using a pre-cleaned sediment grab sampler at about 1 metre and 5 metres from the shore, respectively. The ca.200 g samples were kept in pre-cleaned 250 mls sample jars with lids lined with aluminium foil and kept cooling box kept at low temperatures with dry ice.

Water sampling was done using a pre-cleaned undersurface water sampler about 5 and 10 metres respectively offshore and one metre below the surface. The water samples were kept in pre-cleaned amber coloured 2.5 litre glass bottles. The labeling of samples was done according to type, season and site as shown in the two by two factorial experimental design (Table 4). The samples were then transported and kept in a deep freezer awaiting extraction

Table 4: Experimental design; showing the number of samples per site, season and their labeling.

	Wet season		**Dry season**	
	River Kisat entrance	Car Wash	River Kisat entrance	Car Wash
Sediment samples				
1m off shore	3 (SSa)	3 (SSb)	3 (SSc)	3 (SSd)
5m off shore	3 (SSe)	3 (SSf)	3 (SSg)	3 (SSh)
Water samples				
5m off shore		3 (WSa)		3 (WSb)
10m off shore		3 (WSc)		3 (WSd)
		Total number of samples = 36		

Note: SS - Sediment Sample and the samples were differentiated with lower case letters depending on sampling site and distance from the shore.
WS - Water Sample and the samples were also differentiated with lower case letters as was done with sediment samples.

3.2.2 Preparation of solvents, reagents and glassware

GPR grade methanol, dichloromethane and toluene were all double fractionally distilled before use. Silica gel, glass wool and anhydrous Na_2SO_4 used in the experiments were first put in clean beakers and heated in a Memmert oven at a temperature above 200^0C for at least three hours before being cooled in a dessicator.

Filter papers used for extraction of sediment samples were pre-Soxhlet extracted in the extraction solvent for six hours then kept in a dessicator. All glassware used were soaked in soapless detergent for at least 12 hours. They were then washed and rinsed with tap water followed by distilled water and dried in a Memmert oven at $100^{0}C$ for two hours before being stored in a dust free cabinet. The glassware would always be rinsed with methanol, or acetone, or methylene chloride or n-hexane.

3.2.3 Extraction of analytes from sediment samples

A 50 g mass of each sediment sample was Soxhlet extracted with 250 ml of the double distilled methanol (CH_3OH) for four hours at a fill-empty rate of 2 cycles/hr. The extract was then allowed to cool for 30 minutes and 25 ml of 0.7N KOH added. The resultant mixture was further refluxed for two hours (UNEP, 1992).

The KOH/CH_3OH mixture was then poured into a glass separating funnel with a glass stop cork. The extraction glass was rinsed with two aliquots of hexane totaling to 50 mls and the washings added to the separating funnel. The funnel was tightly stoppered and shaken vigorously for 5 minutes carefully venting vapours out of the inverted funnel periodically, through the tap. The mixture was then clamped on a retort stand and phases allowed to separate. The hexane top layer was then removed and filtered through anhydrous Na_2SO_4 in a Buckner funnel under suction pump and further rinsed with hexane aliquots totaling 20 ml. This layer was the non-saponifiable lipid (NSL) fraction containing the non-volatile hydrocarbons (UNEP, 1992).

The hexane layer was then concentrated to ca. 5ml in a rotary evaporator under an aspirator pump with bath temperature kept at 40^0C. This was kept in a deep freezer awaiting column purification. Purification was done through a 2 cm I.d glass chromatograph column packed with 8 g of deactivated silica gel with Na_2SO_4 added to a height of 2 cm. Two 1ml aliquots of hexane were used to wash the extract onto the silica gel. The PAH were subsequently eluted with 15 ml hexane and then 25 ml of 1:1 (V:V) hexane:dichloromethane mixture. The first 8 ml containing a large amount of aliphatic material was discarded (Christopher *et al.*, 1995). The next eluent containing the analytes was then collected and concentrated under reduced pressure on a rotary evaporator to ca.5ml, then 2 ml of toluene added as keeper (Christopher *et al.*, 1995) and the remainder of hexane and dichloromethane evaporated on a rotary evaporator. The final product was then pipetted into a glass vial and the flask rinsed twice with 1ml washings of toluene and the rinsings added to the sample vial. The samples were then kept in a deep freezer at -4^0C for 4 days awaiting GC-FID analysis.

The analyte was then injected into the GC-17A Ver.3 SHIMADZU under the conditions specified in Table 5

Table 5 : Showing GC-FID conditions for analysis

Column type : DB-17
Column length and ID : 30.0m, 0.25mm ID
Column sampling time : 0.5 min
Column oven program :

Rate	Temperature	Hold time
-	60.0	1.0
3.0	180.0	2.0
5.0	250.0	2.0

Sample size : 5μl
Detector : FID
Detector temperature : 310^{0}C
Flow pressure : 77.0 kPa
Total flow : 3 mL / min
Column flow : 0.83 mL / min
Split ratio : 2
Injection mode : Splitless
Injector temperature : 250^{0}C
Carrier gas : N_2/Air

The GC-FID analysis was done for all the samples, HPLC grade n-hexane (solvent blank), standard mix-9 and surrogate standard. In the GC-FID analysis, n-hexane solvent blank was first injected into the machine and and its chromatogram obtained. This was followed by an injection of the external standard mix 9, them by the injection of the samples. Surrogate standard was then injected to help in the calculation of the recovery percentages.

The external standard mix-9 contained 10ng/L for the individual PAH with expected R.T. The area of every peak was interpreted as representing the amount of the particular PAH (from the observed R.T) in the injected 5μl. This was used to calculate machine response factor for each of the PAH. The R.T from the external standard mix-9 was then used to identify the peaks obtained for the samples and using response factors obtained, the areas of the sample peaks were converted into concentrations. The percent recovery of the surrogate standard was then used to correct the sample concentrations for recovery after the processes of extraction, purification and machine analysis.

3.2.4 Extraction of PAH in water samples

One litre of water sample was put in a 5 litre conical flask lagged with aluminium foil and shaken with 250 ml Dichloromethane on an orbital shaker (SO1 Stuart Scientific model) for 6 hours at 250 rpm (UNEP, 1992). This was then allowed to settle and the lower dichloromethane layer separated in a 500 ml separating funnel. The resultant emulsion layer was removed by filtering through a funnel stacked with glass wool. The dichloromethane was then shaken with 50 g anhydrous Na_2SO_4 and further filtered through glasswool in Buckner funnel (500 ml) under a suction pump. The resultant extract was then concentrated in a rotary evaporator under an suction pump to ca.5 ml and kept in a deep freezer at -20^0C before column purification was done. Column purification and GC-FID analysis followed the same procedure as for the sediment samples.

3.2.5 Preparation of surrogate standard

DNPH (2,4-dinitrophenylhydrazine) was chosen as a surrogate standard as it behaves as most of the PAHs (UNEP, 1992). One gramme of DNPH was dissolved in 5 ml of concentrated H_2SO_4 and diluted with distilled water to 1 litre. The solution was further diluted with distilled water to 1 ppm. One millilitre of the 1ppm DNPH solution was added to 50 g of certified sediment from the National Research Council Canada (Institute of Marine Biosciences) and thoroughly mixed and kept in a dark cabinet for 12 hours before being subjected to the same analytical procedure as the sediment samples. Similarly, 1 ml of the 1 ppm DNPH solution was added to 1 litre of certified standard water, thoroughly mixed, kept in a dark cabinet for 12 hours and then subjected to same analytical procedure as water samples.

These samples with surrogate standards were used to calculate recovery percentage after extraction and purification processes as shown in equation 6 below:

$$\%\ \text{Recovery} = \frac{Amount\ found - Amount\ from\ unspiked\ sample \times 100}{Amount\ spiked} \qquad \ldots\ldots\ldots\ldots\ldots(6)$$

Where:-

Amount found is the calculated concentration from the response of the spiked sample.

Amount from unspiked sample is the original concentration of the spiked sample before spiking.

The recovery ranged between 71 and 78%, showing that the extraction and purification processes did not waste a lot of the analytes (UNEP, 1992). The blank solvent (n-hexane) samples were reduced in a rotary evaporator as the other samples and subjected to GC-

FID analysis. The obtained chromatogram only showed one peak, showing that the n-hexane used was not contaminated by the PAH.

3.2.6 Authentication of results

GC-MS analysis of the external standard mix-9 was done to verify if the compounds detected in the GC-FID analysis were the actual target compounds (PAH). The GC-MS conditions used were as specified in Table 6.

Table 6: Showing GC-MS conditions of analysis

Carrier gas	: N_2
Column	: DB-5, 60m, ID 0.25
Injector temperature	: 280^{0}C, splitless
Initial oven temperature	: 50^{0}C
Final temperature	: 320^{0}C
Injection volume	: 5.00µl
Programme rate	: 4 – 6^{0}C/min

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Determination of types and amounts of PAH in the samples

A number of PAH were detected in the sediment and water samples, with concentrations in the range of 0.04 to 16.46 μg/g for sediment samples and 3.32 to 55.8 μg/L for water samples which is an indication of significant pollution of the Gulf by individual PAH (Tables 7-9). It is important to note that the means were calculated by assuming that sample concentrations below detection limits used were zero. Similar works in other locations have shown that samples of surface water contain individual PAH at levels of up to 50 ng/L, but highly polluted rivers have concentrations of up to 6000 ng/g (WHO, 1998).

Table 7. The mean amounts of identified PAH (μg/g) in Car Wash sediment samples

PAH	Dry season		Wet season	
	1m	5m	1m	5m
Naphthalene	4.72 ± 0.30	6.87 ± 1.82	6.66 ± 0.41	8.51 ± 0.43
Acenaphthylene	nd	nd	nd	nd
Acenaphthene	0.86 ± 0.07	0.93 ± 0.12	0.84 ± 0.04	1.57 ± 0.07
Fluorene	nd	nd	0.04 ± 0.06	nd
Phenanthrene	0.74 ± 0.04	0.75 ± 0.10	1.24 ± 0.23	2.66 ± 0.33
Anthracene	1.42 ± 0.40	1.78 ± 0.11	1.80 ± 0.01	1.76 ± 0.12
Fluoranthene	0.58 ± 0.03	0.68 ± 0.15	0.80 ± 0.04	0.90 ± 0.61
Pyrene	1.10 ± 0.12	1.08 ± 0.26	1.11 ± 0.21	1.54 ± 0.03
Benzo(a)anthracene	nd	nd	nd	nd
Chrysene	0.30 ± 0.04	0.34 ± 0.03	0.95 ± 0.06	1.55 ± 0.40
Benzo(b)fluoranthene	0.44 ± 0.02	0.54 ± 0.01	0.59 ± 0.02	1.63 ± 0.06
Benzo(k)fluoranthene	0.38 ± 0.01	0.42 ± 0.03	0.43 ± 0.03	0.71 ± 0.03
Benzo(a)pyrene	0.87 ± 0.06	1.20 ± 0.49	2.05 ± 0.11	1.05 ± 0.16
Indeno(123cd)pyrene	2.74 ± 0.37	3.25 ± 0.24	2.88 ± 0.25	2.96 ± 0.22
Dibenzo(ah)anthracene	nd	nd	nd	nd
Benzo(ghi)perylene	1.41 ± 0.22	1.60 ± 0.06	2.47 ± 0.08	1.32 ± 0.11
ΣPAH	15.56	19.44	21.86	26.16

Note: 'nd' denotes 'not detected', 1m and 5m refer to sampling distance from the shore.
Mean values and standard deviations are rounded up to 2 decimal points, n=3.

Sediment samples were found to contain relatively higher concentrations of PAH than water samples. The total detected mean concentration for sediment samples had a range of 15.56 to 26.16 and 66.79 to 161.34 μg/g for Car Wash and Kisat sediment samples, respectively, compared to the detected total mean concentration range of 170.44 to 240.65 μg/L (equivalent to 0.17044 to 0.24006 μg/g, taking the density of water as 1 g/cm^3) for water samples. This is consistent with the documented observation that the concentrations of individual PAH in sediment generally occur one order of magnitude higher than those in precipitation (WHO, 1998). Vertical fluxes through the water column are also significantly accelerated in waters by adsorption of both soluble and particulate organic residues into fast sinking fecal materials generated by the zooplankton communities. This phenomenon permits the rapid settling of organic contaminants out of the surface waters and into the sediments (Readman *et al.*, 1984; Burns, *et al.*, 1985; Ko and Baker, 1995). The higher concentrations in the sediment samples compared to water samples, was therefore consistent with these earlier reported observations.

Table 8. The mean amounts of identified PAH (μg/g) in Kisat sediment samples

PAH	Dry season		Wet season	
	1m	5m	1m	5m
Naphthalene	11.77 ± 0.83	15.13 ± 1.03	16.46 ± 1.13	15.62 ± 0.51
Acenaphthylene	5.98 ± 0.71	16.19 ± 1.40	11.39 ± 0.51	17.29 ± 0.34
Acenaphthene	4.18 ± 0.78	8.43 ± 0.29	15.10 ± 1.98	13.85 ±1.48
Fluorene	0.81 ± 0.01	11.44 ± 0.62	13.28 ± 1.10	14.28 ± 0.36
Phenanthrene	6.98 ± 0.47	10.26 ± 0.68	8.25 ± 1.03	9.87 ± 0.08
Anthracene	10.22 ± 0.82	15.33 ±1.39	8.57 ± 0.63	9.52 ± 1.84
Fluoranthene	0.76 ± 0.09	1.62 ± 0.47	4.37 ± 0.51	11.96 ± 0.25
Pyrene	6.39 ± 0.32	15.33 ± 0.36	9.01 ± 0.33	12.22 ± 0.65
Benzo(a)anthracene	2.81 ± 0.30	7.19 ± 0.25	3.59 ± 0.56	nd
Chrysene	2.02 ± 1.53	31.95 ± 1.13	4.21 ± 0.32	19.31 ± 0.32
Benzo(b)fluoranthene	nd	nd	0.28 ± 0.05	1.61 ± 0.15
Benzo(k)fluoranthene	nd	nd	0.27 ± 0.07	nd
Benzo(a)pyrene	1.36 ± 0.25	8.60 ± 0.44	3.40 ± 1.30	7.22 ± 0.82
Indeno(123cd)pyrene	nd	10.53 ± 18.24	0.36 ± 0.13	nd
Dibenzo(ah)anthracene	13.51 ± 0.53	22.83 ± 0.90	16.09 ± 0.91	25.16 ± 2.57
Benzo(ghi)perylene	nd	Nd	3.41 ± 0.46	3.43 ± 0.19
ΣPAH	66.79	174.83	118.04	161.34

Note: 'nd' denotes 'not detected', 1m and 5m refer to sampling distance from the shore.
Mean values and standard deviations are rounded up to 2 decimal places, n=3

Table 9. The mean amounts of identified PAH (µg/L) in Car Wash water samples

PAH	Dry season		Wet season	
	5m	10m	5m	10m
Naphthalene	47.60 ± 5.10	45.26 ± 4.30	50.15 ± 1.45	55.80 ± 1.34
Acenaphthylene	15.66 ± 1.10	28.75 ± 1.14	21.84 ± 1.81	28.67 ± 1.50
Acenaphthene	9.12 ± 1.07	20.76 ± 1.11	13.07 ± 0.54	19.93 ± 0.52
Fluorene	12.08 ± 0.92	9.39 ± 0.58	10.47 ± 1.26	16.14 ± 0.72
Phenanthrene	9.82 ± 0.53	5.39 ± 0.37	3.39 ± 0.18	11.53 ± 1.12
Anthracene	6.47 ± 1.15	4.37 ± 0.66	7.60 ± 0.57	11.78 ± 0.53
Fluoranthene	nd	4.99 ± 0.54	3.53 ± 0.61	5.32 ± 0.26
Pyrene	12.68 ± 0.85	12.57 ± 0.74	11.86 ± 0.74	16.49 ± 0.82
Benzo(a)anthracene	6.58 ± 0.42	7.07 ± 0.48	9.69 ± 1.06	6.85 ± 0.11
Chrysene	5.75 ± 0.47	8.47 ± 1.11	13.87 ± 0.36	8.93 ± 1.53
Benzo(b)fluoranthene	nd	3.32 ± 1.13	4.58 ± 0.38	6.90 ± 1.26
Benzo(k)fluoranthene	9.21 ± 0.96	5.74 ± 0.70	6.82 ± 0.44	9.54 ± 0.83
Benzo(a)pyrene	15.61 ± 0.94	13.82 ± 1.04	10.16 ± 0.97	17.26 ± 0.98
Indeno(123cd)pyrene	nd	nd	nd	nd
Dibenzo(ah)anthracene	7.25 ± 2.10	10.10 ± 0.85	7.10 ± 0.55	9.89 ± 0.11
Benzo(ghi)perylene	14.61 ± 1.17	14.19 ± 1.03	21.94 ± 1.72	15.62 ± 0.71
ΣPAH	172.44	194.19	196.07	240.65

Note: 'nd' denotes 'not detected', 5m and 10m refer to sampling distance from the shore. Mean values and standard deviations are rounded up to 2 decimal points, n=3.

The Kisat sediment samples generally showed higher mean concentrations for the individual PAH than Car Wash sediment samples. This could be attributed to the sediment types from these two sampling points. The Kisat sediments, mainly from deposition by the Kisat river, were coarser and silt-sized while Car Wash sediments were finer. The penetration of oil into sediments is related to the sediment type and composition. Coarser sediments allow greater penetration than fine unconsolidated sediments. Coarser sediments also have higher rates of biodegradation than fine sediments. Consequently, the highest concentrations are associated generally with silt-sized sediments, which have a larger surface area (Yunker *et al*., 1996; Baumard *et al*., 1998; WHO, 1998).

4.2 Concentration variations with seasons and depths.

The mean concentrations were found to exhibit significant differences ($P \leq 0.05$) with seasons and depths, however the degree of significance could not be established in the cases since only two means were compared. The total amount of individual PAH detected in wet season samples was higher than those detected in dry season samples (Figures 4-5). There was significant difference ($P \leq 0.05$) between the mean concentrations for naphthalene, acenaphthylene, chrysene, acenaphthene, phenanthrene, anthracene, pyrene, benzo(k)flouranthene, benzo(a)pyrene and dibenzo(a,h)anthracene detected in wet and dry season Kisat sediment samples.

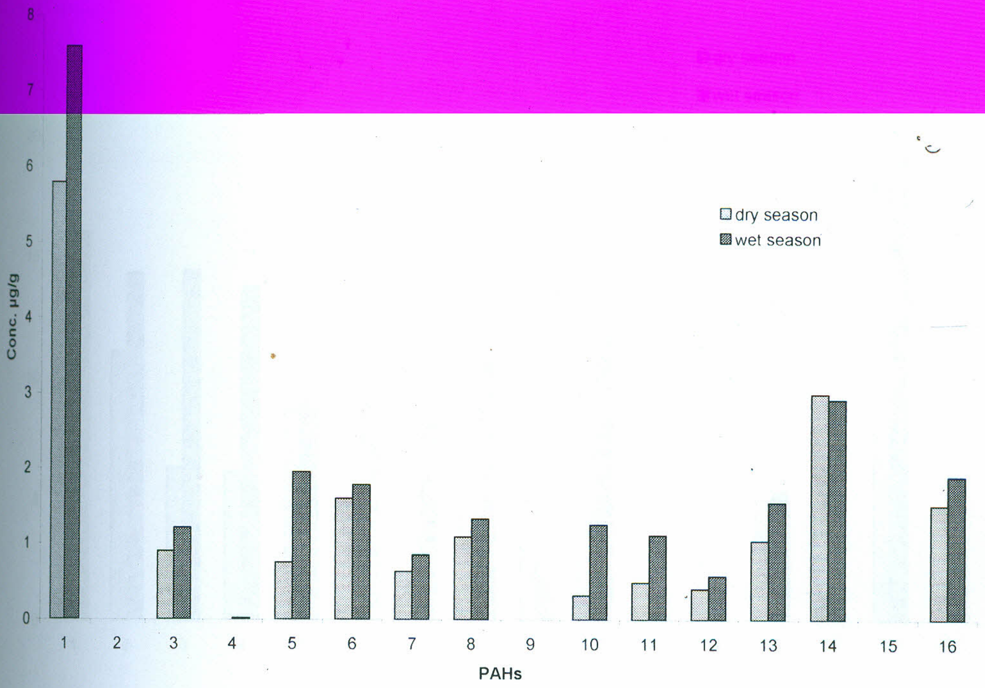


Figure 4: Showing mean seasonal concentration variations for Car Wash sediment samples)

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene,10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene.

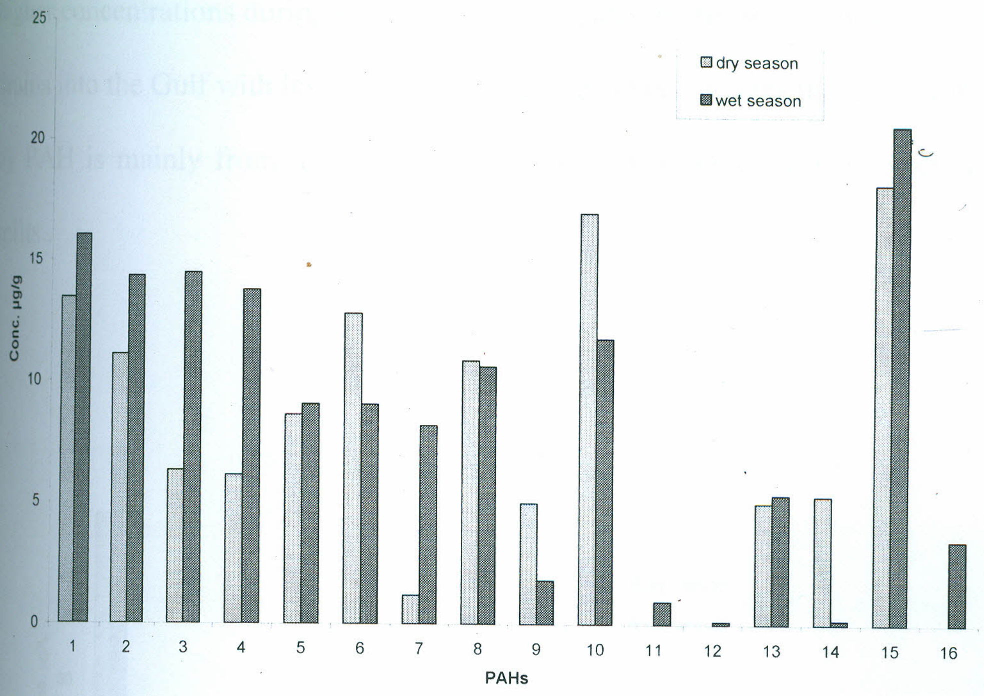


Figure 5: Showing seasonal mean concentration variations for Kisat sediments

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene,10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene.

Car Wash samples also showed significant differences ($P \leq 0.05$) for the mean concentrations of naphthalene , acenaphthylene , acenaphthene , flourene , flouranthene, pyrene, benzo(a)anthracene , chrysene , benzo(b)fluoranthene and benzo(ghi)perylene detected in wet and dry season. This may be attributed to the fact that most of the PAH may be offloading to the gulf through surface run-off from non-point sources such as street runoff, industrial and municipal discharges in addition to the loads derived from the deposition of atmospherically transported residues of combustion and the contaminant loads associated with spills and disposal operations (Boehm, 1987). Surface run-off is higher during rainy seasons and this sweeps through the city's open air garages, open incinerators and sewer systems carrying PAH adsorbed on to solid particles. This leads to higher concentrations during this season as compared to dry season when only Kisat river drains into the Gulf with less PAH pollution load. This indicates that pollution of the Gulf by PAH is mainly from non-point sources rather than point sources such as industrial spills..

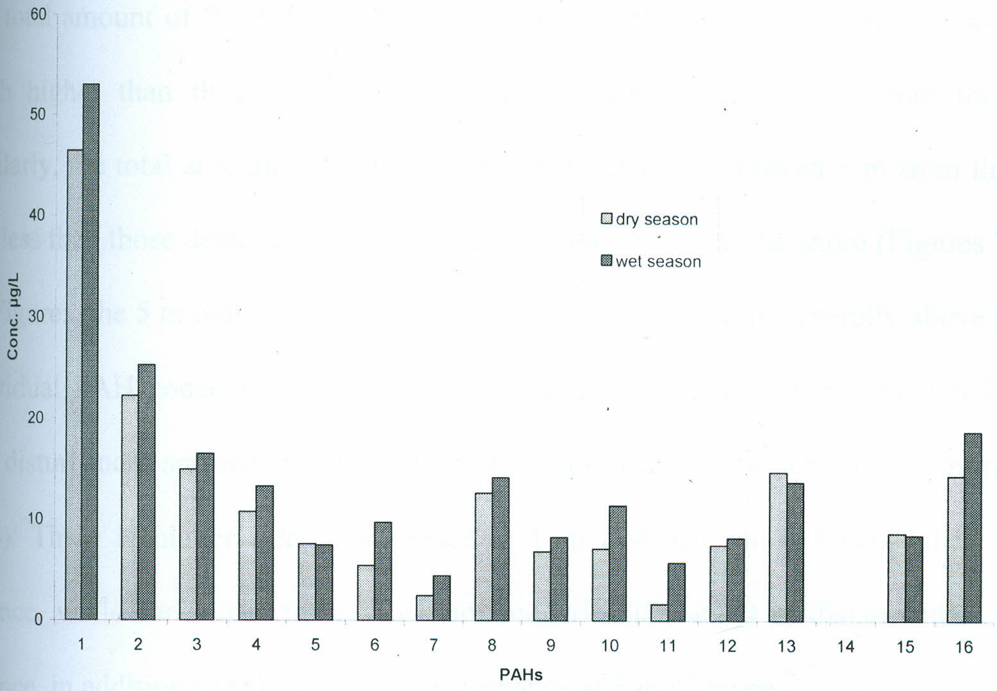


Figure 6: Showing mean concentration variations with season for Car Wash water samples

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene,10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene.

The study also shows that while the sum of mean concentrations of detected PAH in sediment and water samples was notably different, the distribution of individual PAH in the samples was also different. Water samples predominantly accumulated the lower molecular weight PAH; while sediments accumulated the higher molecular weight PAH (Figure 6). This may be explained by the earlier reported observation that higher molecular weight PAH are primarily removed by sedimentation while lower ones through dilution and dissolution (Wakeham, *et al.*, 1980a), these lower molecular weight PAH also tend to be more soluble in water (Wakeham *et al.*, 1980b).

The total amount of PAH detected in sediments samples taken 5 m from the shore was much higher than those detected in sediment samples taken 1 m from the shore. Similarly, the total amount of PAH detected in water samples taken 5 m from the shore was less than those detected in water samples taken 10m from the shore (Figures 7-9). In the Figures, the 5 m individual PAH concentration trend graph is generally above the 1 m individual PAH concentration trend graph. These differences can be explained by the tidal disturbances and redistribution in sediment and water column along the shore (Witt, 1995). There is higher tidal disturbance at 1 m distance for sediments than at 5 m distance, while for water, there is greater redistribution at 10 m distance than at 5 m distance, in addition to enhanced tidal disturbance at 5 m distance.

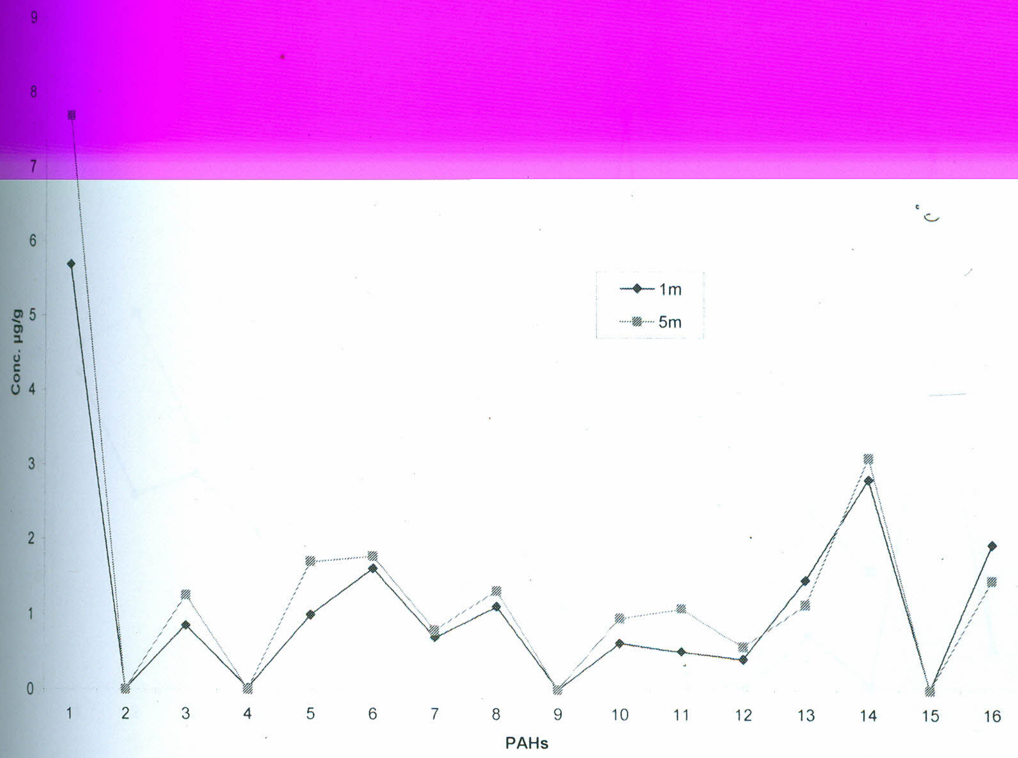


Figure 7: Showing the trend of variations in mean concentration of PAH (1 m and 5 m from the shore) for Car Wash sediment samples.

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene, 10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene.

Note: The individual PAH concentrations are joined to give a comparative trend graph for the two sampling distances

There were significant differences ($P \leq 0.05$) between the mean concentrations for phenanthrene , anthracene , benzo(k)flouranthene and dibenzo(ah)anthracene detected in kisat sediment samples drawn 1 m and 5 m from the shore respectively. Car wash sediment samples had significant differences ($P \leq 0.05$) for the mean concentrations of naphthalene , phenanthrene ,pyrene , chrysene , benzo(a)pyrene and benzo(ghi)perylene

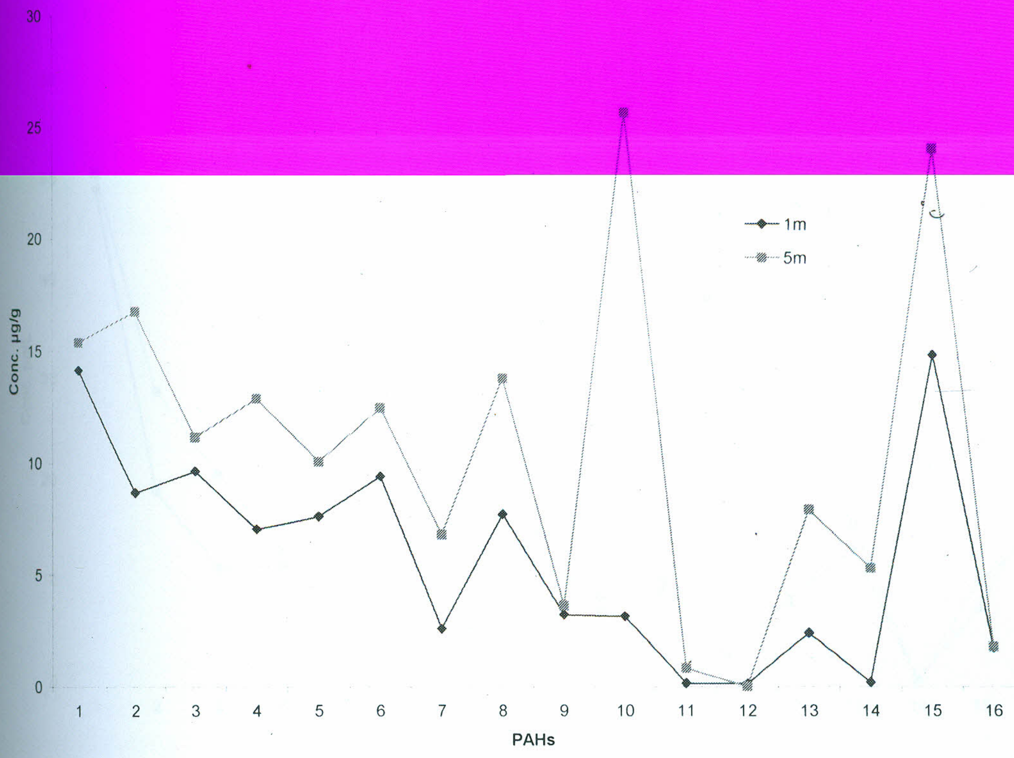


Figure 8: Showing the trend of mean concentration variations of PAH (1 m and 5 m from the shore) for Kisat sediment samples

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene, 10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene.

Note: The individual PAH concentrations are joined to give a comparative trend graph for the two sampling distances

detected in samples drawn 1 m and 5 m off the shore respectively. Similarly, the detected mean concentrations in samples drawn 5m and 10m offshore for water samples showed significant differences ($P \leq 0.05$) for flourene , phenanthrene , anthracene ,pyrene , benzo(a)anthracene , benzo(b)flouranthene ,benzo(a)pyrene ,dibenzo(a,h)anthracene and benzo(ghi)perylene.

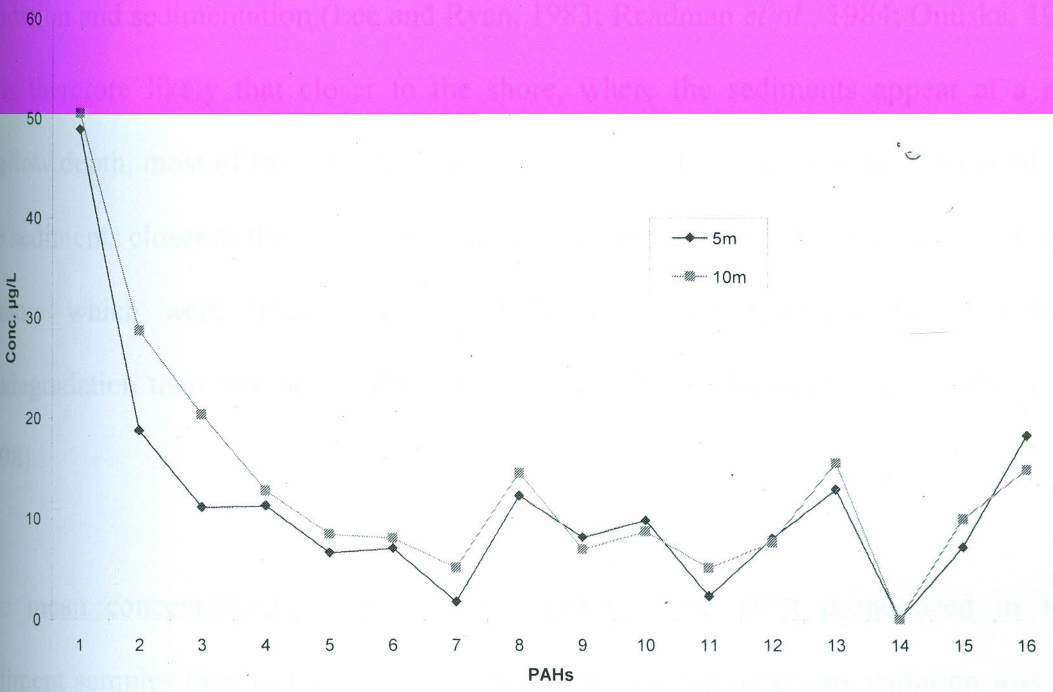


Figure 9: Showing the trend of mean concentration variations of PAH (5 m and 10 m from the shore) for Car Wash water samples

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene, 10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene.

Note: The individual PAH concentrations are joined to give a comparative trend graph for the two sampling distances

This observation is also supported by the fact that there is more light and dissolved oxygen at 1 m distance than 5 m distance. This provides the oxic conditions required for rapid microbial degradation of the PAH (Hinga *et al.*, 1980; Lee and Ryan, 1983; Readman *et al.*, 1984). In the presence of light and oxygen, PAH readily undergo photo-oxidation leading to diols, quinones, aldehydes and dimers as the metabolites. After entering the aquatic environment, organic materials can have various fates, including biological uptake, microbial degradation, volatilization, dissolution and dilution, photo oxidation and sedimentation (Lee and Ryan, 1983; Readman *et al.*, 1984; Onuska, 1989). It is therefore likely that closer to the shore, where the sediments appear at a more shallow depth, most of the PAH transform into their metabolites. It was also notable that the sediments closer to the shore were coarser and silt-sized in contrast to sediments more inshore which were finer in texture. Coarser sediments support higher rates of biodegradation than fine sediments (Yunker *et al.*, 1996; Baumard *et al.*, 1998; WHO, 1998).

The mean concentration variations with distance were most pronounced in Kisat sediment samples than in Car Wash sediment samples. However this variation was least pronounced in Car Wash water samples. This is probably due to variations in depths and perturbation. The Kisat area is rather shallow at 5 m than car wash area due to siltation from the Kisat river. Therefore PAH at 5 m distance in Kisat area are more susceptible to degradation than those from 5 m distance at car wash area perhaps because of greater aeration and higher nutrient concentration (WHO, 1998). Water samples were taken at 1m below the surface both at 5 m and 10 m distances from the shore and therefore the PAH at these two distances were having equal photodegradation effects. The general slight increase in the mean concentrations between 5 m and 10 m distances for water samples could be attributed to the fact that there is more disturbance of the PAH at 5 m distance by the waves than at 10 m distance from the shore. PAH generally adsorb to suspended particles in surface waters with the lighter hydrocarbons remaining in solution (Boehm, 1987). Sorption of organic contaminants onto particles has been shown to depend not only on the particulate loading, but also on the types and degree of aggregation of the particles available. This is greatly influenced by the wave disturbances. The lipid particles onto which PAH are more adsorbed are more dispersed in areas that experience higher disturbances (Dixon *et al.*, 1987).

The concentration variations could also be attributed to distance and season interactions as these interactions showed significant differences as indicated by the given LSD values (Figures 10-12). This could be because there is less variation in mean concentrations as the distance increases in shore, even with seasonal variations. This observation may be attributed to dilution factor on the PAH concentrations which tends to become constant with increase in depths (WHO, 1998). It is therefore possible that the dilution fate of the PAH is the most dominant factor making the sediments inshore to have relatively lower constant PAH concentrations.

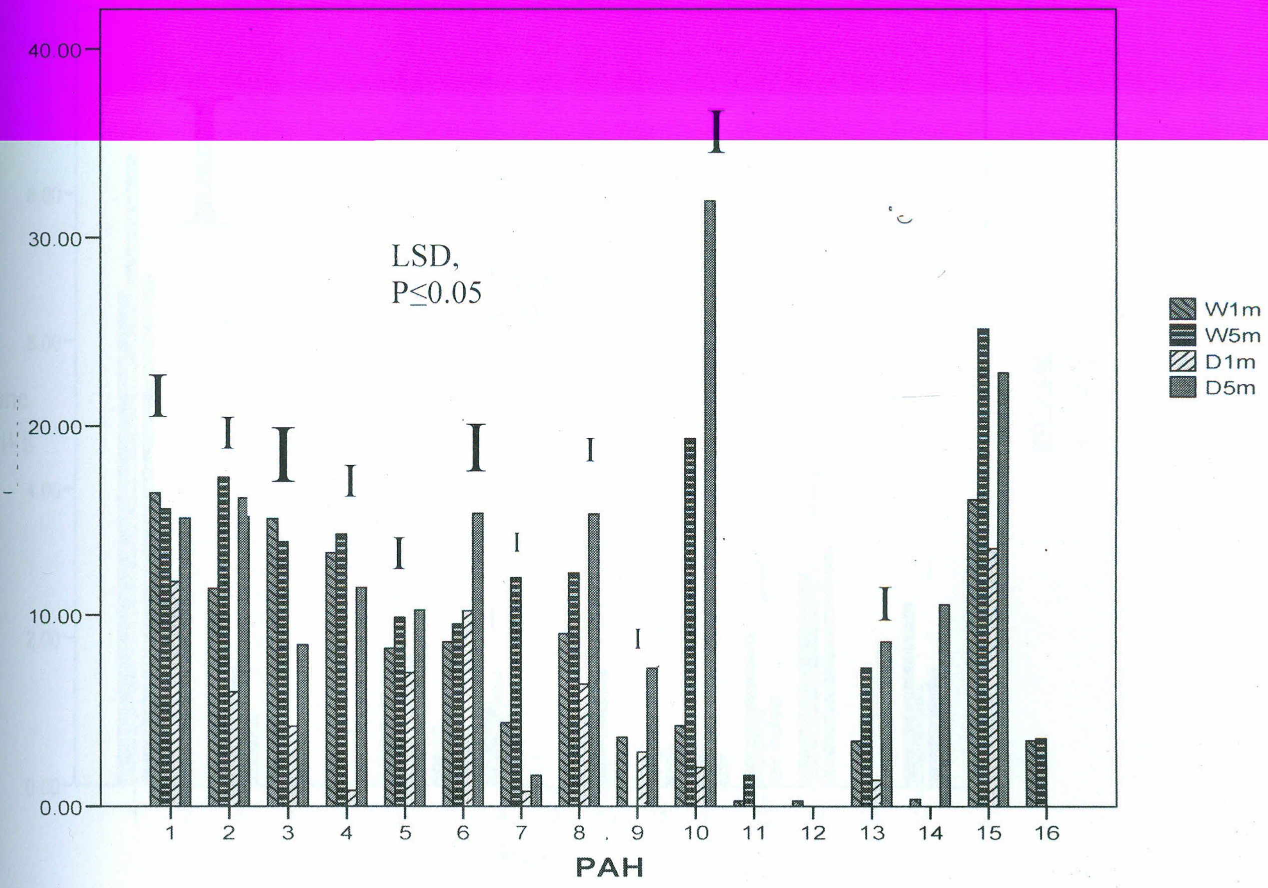


Figure 10: Kisat sediment season-distance mean PAH concentrations graph

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene, 10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene.

(I)-LSD values

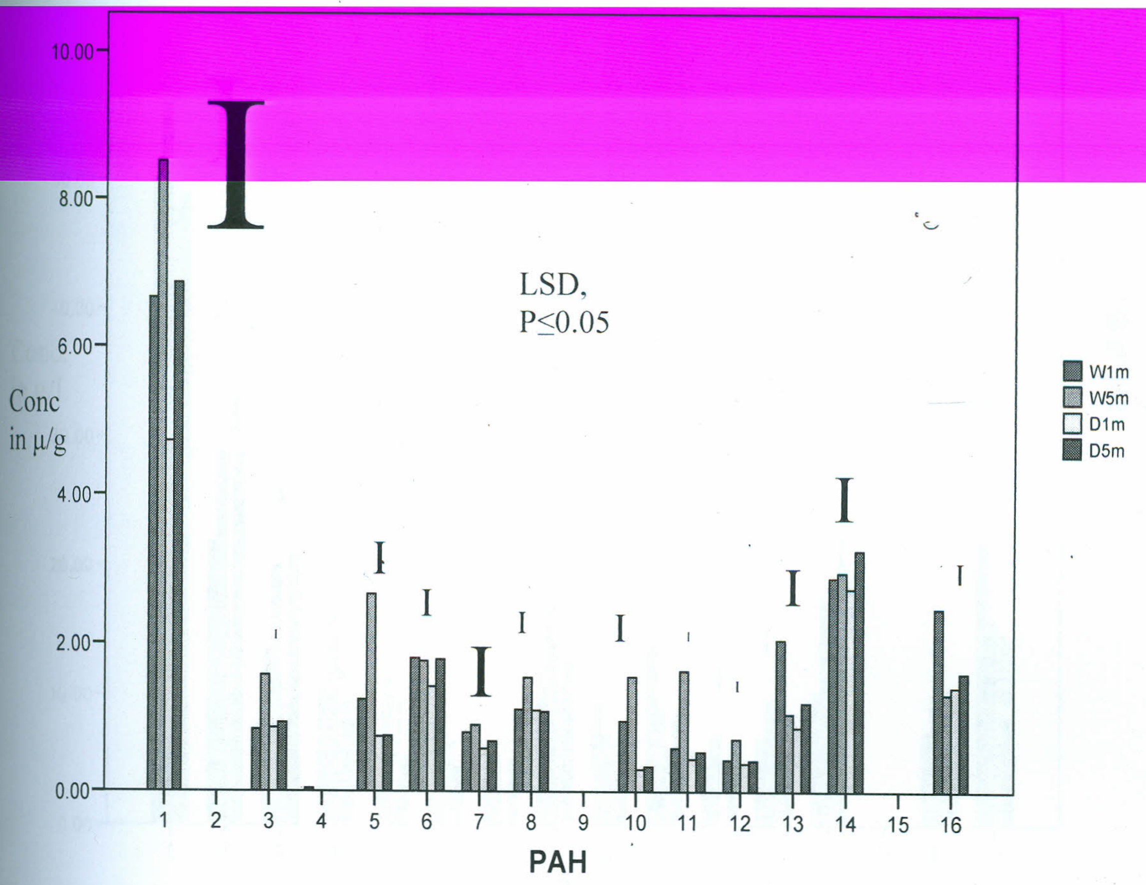


Figure 11: Car Wash sediment season-distance mean PAH concentrations graph

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene, 10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene. (I)-LSD values

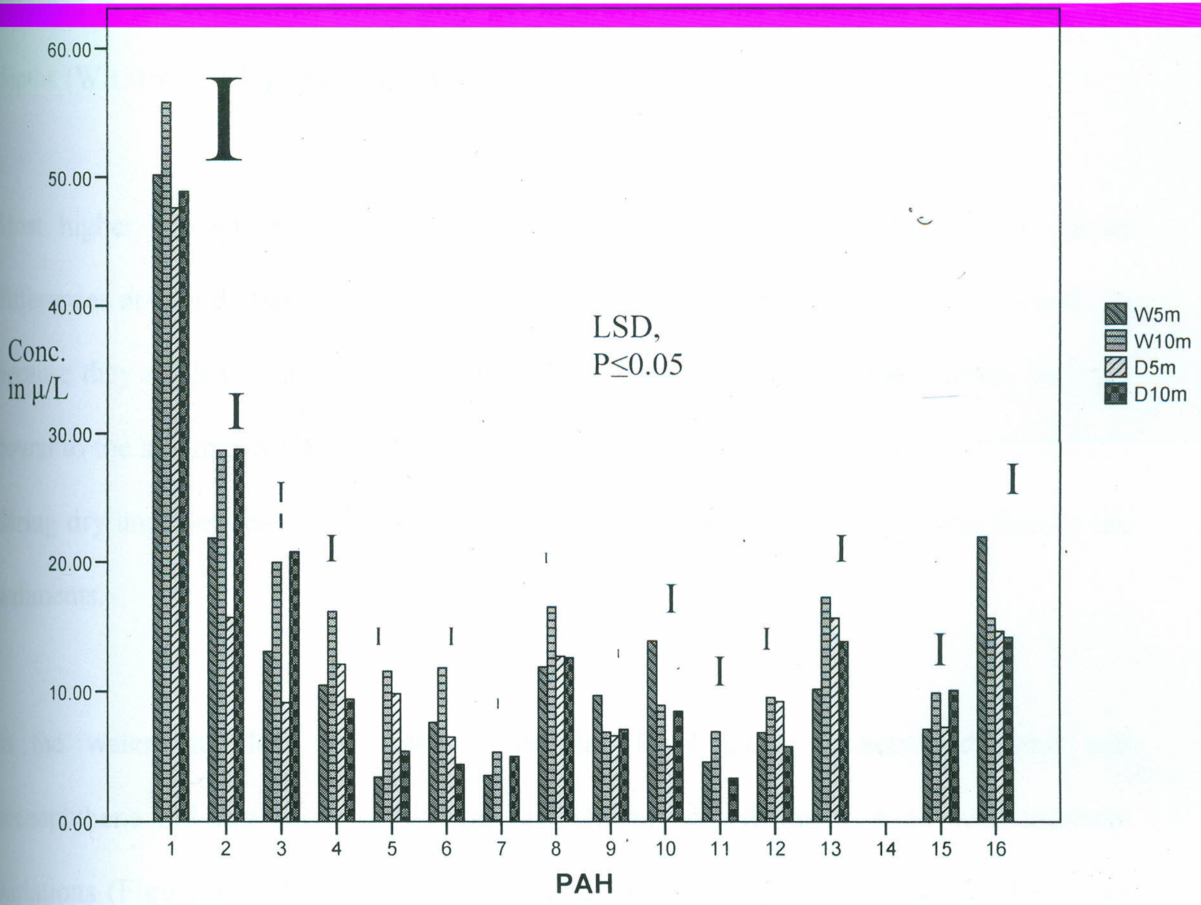


Figure 12: Car Wash water season-distance mean PAH concentrations graph

Key: 1-naphthalene, 2-acenaphthylene, 3-acenaphthene, 4-fluorene, 5-phenanthrene, 6-anthracene, 7-fluoranthene, 8-pyrene, 9-benzo(a)anthracene,10-chrysene, 11-benzo(b)fluoranthene, 12-benzo(k)fluoranthene, 13-benzo(a)pyrene, 14-indeno(1,2,3-c,d)pyrene, 15-dibenzo(a,h)anthracene, 16-benzo(g,h,i)perylene. (I)-LSD values

Most lower molecular weight PAH like naphthalene and acenaphthylene only showed significant differences 1 m from the shore and not 5 m from the shore for sediment samples, even with changes in seasons. This could be attributed to their being more soluble in water and therefore do not easily settle to the bottom of the water to get adsorbed to the sediments, hence only get in touch with the sediments at more shallow depths (Wit, 1995; Ying *et al*, 2003).

Most higher molecular weight PAH like Chrysene and pyrene showed significant differences at 5 m distances from the shore with seasonal variations (Figures 10 and 11) because they are less soluble and tend to settle at the bottom of the water where they get bound to the sediments (Wit, 1995; Ying *et al*, 2003). The amounts that enter the waters during dry and wet seasons would therefore affect the amounts that get adsorbed to the sediments.

In the water samples, the lower molecular PAH such as acenaphthylene and acenaphthene did not show significant differences with distance, even with seasonal variations (Figure 12). This was also observed with higher molecular weight PAH like benzo(a)anthracene, chrysene and benzo(ghi)perylene. This could be attributed to the fact that for water sample, dispersion in water for the PAH is usually very fast as a result of waves, and that solubility of the individual PAH would not change with season (Witt, 1995; Ying *et al*, 2003).

4.3 Detected pollution levels versus some exposure limits set by different Environmental Safety Bodies

The average individual PAH accumulation levels in the Car Wash water samples were found to be several magnitudes above the recommended exposure limits by various bodies. The National Institute for Occupational Safety and Health (NIOSH) recommends an exposure limit of 0.1 mg/cm^3 of air averaged for a ten hour workday within a forty hour workweek. American Conference of Governmental Industrial Hygienists (ACGIH), gives an exposure limit of 0.2 mg/cm^3 for an 8 hour workday, within a 40 hour workweek. Similarly, Occupational Safety and Health Administration (OSHA), limits the exposure to individual PAH at 0.2 mg/cm^3 (ATSDR, 1995).

The average accumulation ranges for individual PAH detected in the Car Wash water samples, within the used parameters of season and distance from the shore, were as shown in Table 10.

Table 10. Showing average individual PAH accumulation ranges and weekly exposure to PAH on consumption of Car Wash water

PAH	Range (µg/L)	Weekly exposure for a 2 L consumption per day (mg)
Naphthalene	45.26 - 55.80	0.63 – 0.78
Acenaphthalene	15.66 - 28.75	0.22 – 0.40
Acenaphthene	9.12 - 20.76	0.13 – 0.29
Fluorene	9.39 - 16.14	0.13 – 0.23
Phenanthrene	3.39 - 11.53	0.05 – 0.16
Anthracene	4.37 - 11.78	0.06 – 0.16
Fluoranthene	3.53 - 5.32	0.05 – 0.07
Pyrene	11.86 - 16.49	0.17 – 0.23
Benzo(a)anthracene	6.58 - 9.69	0.09 – 0.14
Chrysene	5.75 - 13.87	0.08 – 0.19
Benzo(b)fluoranthene	3.32 - 6.90	0.05 – 0.10
Benzo(k)fluoranthene	5.74 - 9.54	0.08 – 0.13
Benzo(a)pyrene	10.16 - 17.26	0.14 – 0.24
Indeni(123-cd)pyrene	not detected	
Dibenzo(ah)anthracene	7.10 - 10.10	0.10 – 0.14
Benzo(ghi)perylene	14.19 - 21.94	0.20 – 0.31

Note: Data includes 'dry' and 'wet' seasons.

The concentration levels detected are comparable to the weekly exposure limits given by NIOSH (0.1 mg/L of air), ACGIH (0.2 mg/L of air) and OSHA (0.2 mg/L of air). Generally, exposure to PAH from Car Wash is higher that these limits, an indication that Car Wash water is significantly polluted by PAH.

Environmental Protection Agency (EPA), has also given lower limits on carcinogenic accumulation levels for some PAH per kg body weight as shown in Table 11 (ATSDR, 1995). Above these levels given by EPA, the individual PAH are carcinogenic. It therefore means that for an average body weight of 55kg, the accumulation limits in μg would be 16500, 3300, 2200, 2200 and 1650 for anthracene, acenaphthene, fluoranthene, fluorine and pyrene respectively. A person of 55kg body weight taking 6 litres of Car Wash water per day would therefore reach the anthracene carcinogenic lower limit within 234 days, assuming there are no processes removing it from the body. A similar assumption would give 27, 69, 23 and 17 days for acenaphthene, fluoranthene, fluorine and pyrene, respectively. This would indicate very high toxic exposure levels.

Table 11: Lower carcinogenic accumulation limits per kg body weight

Anthracene	0.3 mg
Acenaphthene	0.06 mg
Fluoranthene	0.04 mg
Fluorene	0.04 mg
Pyrene	0.03 mg

Source: ATSDR, 1995

4.4 GC-MS authentication results

Based on GC-MS online library, the GC-MS identified the PAH at different retention times as shown in table 12

Table 12: Showing the GC-FID and some GC-MS retention times for the identified PAH

PAH	**GC-FID retention time (mins)**	**GC-MS retention time (mins)**
Acenaphthene	18.61	17.55
Acenaphthylene	16.52	16.55
Anthracene	26.96	26.05
Benzo(a)anthracene	40.01	nms
Benzo(b)fluoranthene	44.52	44.26
Benzo(k)fluoranthene	44.85	nms
Benzo(ghi)perylene	54.25	nms
Benzo(a)pyrene	45.51	45.56
Chrysene	41.49	nms
Dibenzo(ah)anthracene	53.25	nms
Fluoranthene	31.63	31.55
Fluorene	20.46	20.50
Indeno(1,2,3-c,d)pyrene	51.79	51.41
Naphthalene	13.23	nms
Phenanthrene	25.99	25.50
Pyrene	33.42	nms
2,4-DNPH	28.47	nms

Note: nms-not detected by GC-MS

The GC-FID retention times were quite comparable with those of GC-MS indicating that the GC-FID method of analysis was appropriate. The GC-FID method can therefore be good for analysis for samples expected to be highly polluted since its detection limit is appreciably high, However, when used, GC-MS must be used to confirm if the detected compounds are the target compounds since GC-FID is not confirmatory.

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the two sampling points within Winam Gulf, i.e. Car Wash and Kisat river mouth, this study has identified the sixteen USEPA characterized carcinogenic PAH and determined the extent of pollution of the Gulf region by these contaminants. In all the cases where these PAH were detected, the pollution levels by individual and total PAH were found to be significant. Considering the data presented in this report and those presented elsewhere in the literature, the conclusion drawn in this study can be summarized as below:

1. The detected PAH in the water and sediment samples were in the μg/g range, which is a significant contamination level by the individual PAH. Only Acenaphthalene, Benzo(a)anthracene and Dibenzo(a,h)anthracene were not detected in Car Wash sediments. Similarly, indeno(1,2,3-c,d)pyrene was not detected in the Car Wash water samples within the GC-FID detection limits.of 0.2-10 ng/g for sediments.
2. The sediment samples were found to contain higher mean individual and total PAH concentrations than water samples drawn from the same sampling points.
3. Kisat sediment samples exhibited higher mean concentrations for individual and total PAH than Car Wash sediment samples indicating that runoff is a major source of PAH pollution to the Gulf.
4. Lower molecular weight PAH e.g. Naphthalene were found to have higher mean concentrations in water samples compared to higher molecular weight PAH e.g Chrysene.

5. Wet season samples showed higher mean concentrations for individual and total PAH than dry season samples both in Car Wash and Kisat sampling points.
6. Individual and total mean PAH concentrations generally increased with increasing distance inshore, both for sediment and water samples.
7. The mean concentrations of individual PAH showed less variation with season with the increase of sampling distance inshore. This difference could be attributed to water disturbances nearer the shore.
8. Pollution levels of the sampled Car Wash water by individual PAH were found to be far above the acceptable levels given by EPA, NIOSH, OSHA and ACGIH. The use of this water for drinking and domestic cooking can be a significant source of human exposure to PAH.

5.2 Recommendations

1. Further work on accumulation levels of individual and total PAH in fish and other biota from the Gulf region as these can be important sources of PAH exposure to the dependant human population.
2. It is clear as reported in this work that pollution of the Gulf by PAH is through urban runoff, it is recommended that the City council of Kisumu should formulate by-laws restraining oil companies operating filling stations from direct disposal of oil and oil products into open sewers.
3. It is also recommended that Car Wash activities be restricted to built up sheds with oil receptors to avoid oil spills finding its way to the Gulf waters. Similarly open air garages should be reconstructed to include oil receptors for used oil disposal.

4. The Kisumu Water and Sewerage Company should consider treatment of sewers before discharge to the lake. Currently the Kisat river more or less discharges raw sewers into the waters of the Gulf. The treatment of water for domestic use should also include reduction of levels of total PAH pollutants.
5. Further work should be done on other sites within the Gulf to establish the types and quantities of PAH in these sites as various points of the Gulf may be exposed to PAH through different environmental processes.
6. Further work need also be done on the Gulf to establish the presence of other carcinogenic PAH, their substituted forms and their metabolites as it is believed that a good number of PAH convert to their metabolites in oxic conditions, and that some metabolites are more carcinogenic than the parent PAH. The investigation of the PAH transformation and the metabolites formed should also be done.

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