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## TOTAL PETROLEUM HYDROCARBONS IN ORGANS OF COMMERCIALLY AVAILABLE FISH; *TRACHURUS TRECAE* (CADENAT, 1949) FROM OLIHA MARKET, BENIN CITY, NIGERIA.

Enuneku, A. A<sup>1</sup>., Ainerua M<sup>2</sup>., Erhunmwunse, N. O<sup>3</sup>., Osakue O. E<sup>4</sup>.

<sup>1</sup>Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria. <sup>234</sup> Laboratory for Ecotoxicology and Environmental Forensics, Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria. Corresponding Author: alex.enuneku@uniben.edu (Received: 18th May, 2015; Accepted: 23rd June, 2015)

#### ABSTRACT

Total petroleum hydrocarbons (TPH) in a commercially available fish *Trachurus trecae* from Oliha Market, Benin City Nigeria were assessed. *Trachurus trecae* is imported into Nigeria and consumed in Benin City. A total of nine fishes were purchased over a period of three months. Samples were measured, prepared and dissected to obtain the liver, gills, muscle and kidney. The samples were analyzed using a Gas Chromatograph-Mass Spectrophotometer. The results obtained indicated that the mean total aliphatic hydrocarbons (mg/kg) ranged from  $47.98\pm0.33-92.56\pm0.62$ ,  $121.97\pm0.81-225.74\pm1.05$ ,  $20.72\pm0.14-38.38\pm0.26$ ,  $43.88\pm0.29-81.27\pm0.54$  for liver, gills, muscle and kidney respectively. The mean  $\Sigma$  poly aromatic hydrocarbon (mg/kg) ranged from  $2.11\pm0.01-3.91\pm0.03$ ,  $5.15\pm0.03-9.54\pm0.06$ ,  $0.88\pm0.01-1.62\pm0.01$  and  $1.86\pm0.01-3.44\pm0.02$  for liver, gills, muscle and kidney, respectively. Total petroleum hydrocarbon had mean concentrations of 73.31, 178.82, 30.40 and 64.37mg/kg in liver, gills, muscles and kidney. TPH concentrations showed a trend: gills > liver > kidney > muscle. The mean concentration in the fish muscle studied (mostly consumed by humans), though was low compared to other organs analyzed, but was greater than EU recommended benchmark of  $2\mu g/kg$ ; wet weight for fish. Periodical monitoring and assessment of TPH contamination and its bioaccumulation in marine organisms is recommended from public health view point.

Keywords: Trachurus trecae, Organs, GC-MS, Total Aliphatic Hydrocarbons, Poly Aromatic Hydrocarbon

### INTRODUCTION

The contamination of aquatic ecosystem by natural and anthropogenic hydrocarbon inputs is attributable to industrial and maritime activities. These increased activities have resulted in extensive environmental pollution by oil spills involving blowouts, leakages from tanks or tanker trucks and dumping of waste petroleum products into the environment (Benson et al., 2007). Continual oil spillage has led to contamination of aquatic and terrestrial environments worldwide (Al-shwafi 2008). The coastal ecosystem and its fishery resources are particularly at high risk of exposure to petroleum hydrocarbon during large oil spills (Burt et al., 1992; Halpern et al., 2008). Long term but low addition of oil into the marine environment from shipping, de-ballasting and other anthropogenic activities is absorbed by marine organisms because of its low degradability and persistence (Vandermeulen et al., 1985). The accidental discharge of hazardous materials such as petroleum and chemical solvents to the aquatic environment has become the focus of increasing regulatory and public concern because of the adverse impacts of such materials on human health and the environment (Ritschard *et al.*, 1981; Bourodimos and Carvoumis, 1990).

Total petroleum hydrocarbon (TPH) is defined as the measurable amount of petroleum-based hydrocarbon in an environmental media. They are called hydrocarbons because they are compounds of hydrogen and carbon only. The amount of TPH found in a sample is useful as a general indicator of petroleum contamination at that site. TPH is released to the environment through accidents, as releases from industries, or as byproducts from commercial or private uses. When TPH is released directly to water through spills or leaks, certain TPH fractions will float in water and form thin surface films. Other heavier fractions will accumulate in the sediment at the bottom of the water which may affect bottomfeeding fish and organisms. Some organisms

found in the water (primarily bacteria and fungi) may break down some of the TPH fractions. Since hydrocarbons are found to be carcinogenic, eventually this will pose severe health problems to humans should they consume organisms contaminated by hydrocarbons (Connell *et al.*, 1980; 1981).

In recent years, there has been an increasing interest in the utilization of fishes as bioindicators, of the integrity of aquatic environmental systems (Fausch et al., 1990). The response of fish to environmental change makes it suitable for use as an indicator for environmental pollution (Batvari et al., 2007). Despite the numerous benefits of fish as fish diet, the potential health risk arising from frequent consumption of fish is a great concern. All fish ingest petroleum hydrocarbons directly or indirectly from contaminated water as food and sediments leading to massive destruction of aquatic biota (Asuquo and Ewa-Oboho, 2004). Both of the aliphatic and polycyclic aromatic hydrocarbon fractions of dissolved petroleum are readily absorbed by most finfish and shellfish because of their high lipid solubility and are bioconcentrated in them (Gobas et al., 1999).

The aliphatic and polycyclic aromatic hydrocarbon fractions of dissolved petroleum are readily absorbed by most aquatic organisms because of their high lipid solubility and are bioconcentrated in fish and shellfish (Gobas *et. al.*, 1999). Oil may enter fish through the skin or gills (Al-Zarouni, 1997).

The Food and Agriculture Organization (1994) asserted that fish contributes about 60% of the world's supply of protein and that 60% of the developing world derives more than 30% of their annual protein from fish (Amusan *et al.*, 2010). However, in Nigeria, fish constitute 40% of the animal protein intake (Olatunde, 1998; Amusan *et al.*, 2010). In Nigeria, there is high demand for fishery products arising from the awareness of its significance in the local diet and its favorable price compared to its substitutes (Agbebi, 2010). Also the rapid awareness that fish is rich in nutrient contributing an average of 20-25% of per capita animal protein intake and could be as high as 80% in coastal and riverside communities (FAO, 2000).

*Trachurus trecae* is bentopelagic (a schooling species), usually occurring near bottom  $(15 - 22)^{\circ}$ C) between 20 and 100 m depths; also sometimes pelagic and near surface at times. Its binomial name is *Trachurus trachurus* and it is a species of the *Carangidae* family. It gets its common name from the legend that other smaller fishes could ride on its back over great distances. Other common names include Common Scad, Maasbanker Pollock, Saurel and rough Scad.

Several studies have reported the negative effects of petroleum hydrocarbon to human health (Nkpaa et al., 2013; Rose et al., 2012; Asuquo and Ewa-Oboho 2004). Recently, studies have shown that most human cancers such as prostrate and lung cancer can be attributed to dietary sources (Dhananjayan et al., 2012; Shen et al., 2008). In the long term, the sub-lethal dose of oil and oil products have produced physiological and histopathological changes (Anderson et al., 1974; Mazhar et al., 1987) that can affect marine life by interfering with the ability to breed, reproduce, grow, or perform other vital functions. In extreme cases, death and development of genetic mutation in fish, shellfish, marine mammals, reptiles and birds may occur.

Accumulated aliphatic and polycyclic aromatic hydrocarbon fractions of dissolved petroleum can harm marine life, both in the long and short term. Small quantities of crude oil mixed with seawater have been shown to affect the feeding behaviour of fish and shellfish (Al-shwafi 2008).

If information about level of contaminants in the environment is available, it is possible to estimate contaminants of the level by comparing it with international standard values and ultimately find a way to reduce its unfavorable levels (EPA, 2003), although risks to human health, due to presence of petroleum hydrocarbons are not well documented, the possible consequences of bioaccumulation should not be ignored especially in communities consuming large quantities of fish.

This work was done to access the level of TPH and possible health risk in the consumption of cold room fish, *Trachurus trecae* sold in Oliha market, Benin City.

### **MATERIALS AND METHODS**

#### Sample Site

The traditional Oliha market in Benin City lies on Latitude: 60 22'N and Longitude: 50 34'E, is one of the few major markets in Benin City known to sell rare animals, native chalks, coins and several other materials which have spent over two hundred years. Feathers of rare birds like ostrich; sparrow and even vulture, all of which have different connotations are sold here. However, other general household and kitchen items for general consumption are also sold

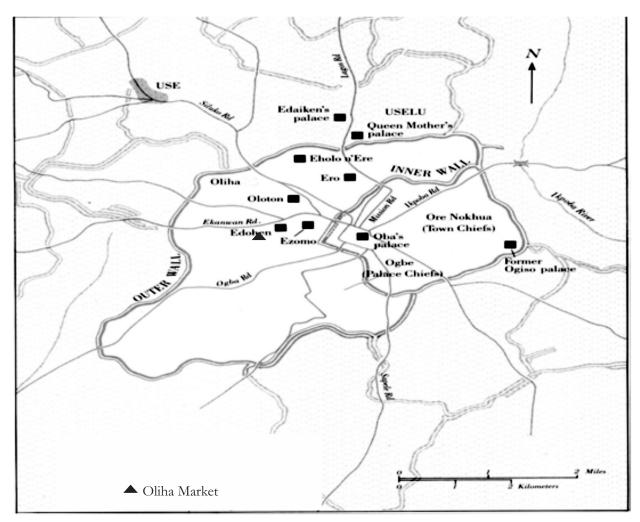


Figure 1: Map of Benin City showing Oliha market

### Sample Collection

A total 9 Fishes of *Trachurus trecae* were collected over a period of August to October 2014 from Oliha market in Benin City. The samples were taken to the laboratory for proper identification, measured and weighed. The length of the samples ranged from 30–35cm while their weight measured between 386.90 – 588.20g. The samples were then prepared for further analysis.

### **Sample Preparation**

In the laboratory, the samples were removed from

the ice pack, thawed, and cleaned in tap water to remove any dirt. They were dissected using aseptic instrument and dishes to obtain the liver, gills, muscles and kidney and placed in a sample bottle. The samples were then labeled.

## Sample Processing and Total Petroleum Hydrocarbons Extraction

Each fish sample was cut into pieces and crushed in a mortar with pestle. Ten grams of individual fish sample was weighed using analytical balance into a 100 mL beaker and 60mL of acetone and dichloromethane (1:1 v/v) was used as the extracting solvent. The fish TPH contents were extracted by a shaking method based on Schwab et al (1999). The beaker with the content was placed on magnetic stirrer/ heater and shaken for about 10 minutes at 70°C. The extract was decanted into a clean round-bottom flask. Then 30 mL fresh solvent was added and the process repeated. The extracts were combined and 5 g of anhydrous sodium sulphate was added to remove water. The extract was concentrated to 3 mL with rotary evaporator maintained at 20 °C. Then 1.5 mL of the concentrated extract was loaded on a silica gel column. The silica gel column was prepared by loading a 2 g glass wool followed by 30 g silica gel, onto a chromatographic column (2 cm internal diameter and 10 cm long). Each of the beds was conditioned with 40ml HPLC-hexane to remove any organic contaminant. The 1.5 mL concentrated extract was loaded and eluted with 30 mL HPLC hexane into a labeled 100 mL beaker to get the aliphatic hydrocarbon components in the sample. After the hexane had almost eluted through the column, but before completely letting the column dry, 30 mL of dichloromethane was added to elute the aromatic hydrocarbon contents into another labeled 100mL beaker. Then 2 g of anhydrous sodium sulphate was added to remove any traces of water left in the extract. The fractions were concentrated using rotary evaporator to about 2ml. Then 1ml of the extract was transferred into a well labeled vial ready for gas chromatographic analysis. The samples were stored at 4°C until GC analysis.

### Gas Chromatographic Analysis

Each extract transferred to 1.5 mL vial was loaded into a gas chromatography system Agilent 6890 series model G1530 A, with flame ionization detector (FID), and cold on-column injection. 1µL portion of the sample was injected and analyzed for TPH (C9–C36). A HP-5 (crossslinked PH ME siloxane) column having the dimensions 30m x 0.25mm 1.d with a stationary phase thickness of 0.25µm was used for analytical separation. The carrier gas was purified nitrogen held at a flow rate of 5mL/min. The operating temperature program was started at 60°C for 2mins and then increase at a rate of 10°C/min to 300°C for 10min (API 1968). The injector and detector temperature were maintained at 250°C and 300°C respectively. The minimum detection limit for all the compounds analyzed was 0.1  $\mu$ g/kg wet weight.

# QUALITY ASSURANCE/QUALITY CONTROL

For the Standard solution, A standard stock solution of 1mg/ml was prepared in HPLC grade Dichloromethane (DCM) and stored at 2-8°C. Standard solution was prepared daily using the appropriate dilution method.

In the Preparation of internal standard stock solution, 1ml of n-hexane was taken and transferred into 100ml volumetric flask, dissolved and diluted to volume using HPLC grade DCM. In the Preparation of reference standard solution, 100mg RSTPH was pipetted into 100ml volume flask and 10ml of 1% v/v n-hexane was added. Then the solution was made to mark with HPLC grade DCM with concentration 1mg/ml.

For method validation specificity (selectivity), the selectivity of the GC method was checked by comparison of chromatograms obtained from samples and the corresponding standard. For linearity, the linearity was determined by plotting a calibration curve vs detector response from the reference standard stock solution of TPA containing Hydrocarbon at concentration level 100mg/ml aliquots.

The calibration curve obtained was subjected to statistical analysis to calculate the calibration equation and the correlation coefficient (r). The test fit was achieved by a linear regression equation of TPH.

The precision of the method was determined in terms of repeatability or reproducibility and intermediate precision studies. The accuracy of the method was evaluated by spiking different known concentration of hydrocarbon into the gas chromatograph and the closeness of the results to the true value was determined.

### **Statistical Analysis**

All data were subjected to one-way Analysis of Variance (ANOVA) using SPSS version 16 to test for the significant level of the parameters across the canned groups. The level of significance was chosen at P<0.05 and the results were presented as mean  $\pm$ standard error. Graphical representation was used for further illustration.

## RESULTS

Tables 1, 2, and 3 show mean concentrations (mg/kg) of individual aliphatic and aromatic hydrocarbon contents in sampled fish species from August to October. The results for aliphatic and aromatic hydrocarbons showed a progressive decrease in mean values from August to October. This is ultimately reflected in the mean total petroleum hydrocarbon (TPH). Within the various organs analyzed, Analysis of Variance (ANOVA) showed that the difference in the mean concentration of the components of the aliphatic hydrocarbons, aromatic hydrocarbons and total petroleum hydrocarbons were highly significant (P<0.001).

### Total Aliphatic Hydrocarbon (TAH)

TAH is made up of components of n-alkanes. The components of aliphatic hydrocarbon analyzed showed values that varied across the months. The gills had the highest concentrations (mg/kg) across the various months with values ranging from  $11.97\pm0.08-22.17\pm0.15$  for Nonane (C9). Decane (C10) had a mean value that ranged from  $24.42\pm0.16-45.23\pm0.30$ . Others include; Dodecane (C 12)  $30.37\pm0.20-56.23\pm0.37$ , Tetradecane (C 14)  $19.35\pm0.13-35.83\pm0.24$ . Hexacosane (C 26) with values ranging from  $0.94\pm0.01-1.74\pm0.01$  had the least levels in the

gills. Besides, the mean total aliphatic hydrocarbon recorded for gills ranged from  $121.90\pm0.81$ - $225.74\pm1.50$ .

The least values for aliphatic hydrocarbon across the sampled period were among the muscles which had a mean total aliphatic hydrocarbon ranging from  $0.24\pm0.002$ - $0.44\pm0.003$ . The Liver and Kidney showed similar results. Their mean total aliphatic hydrocarbon values ranged from  $47.98\pm0.33$ - $92.56\pm0.62$  and  $43.88\pm0.29$ - $81.27\pm0.54$ .

### Poly Aromatic Hydrocarbon (PAH)

The results for aromatic hydrocarbon showed that the gills of *Trachurus trecae* recorded the highest mean values across the months sampled. The values decreased down the month from August to October with a mean value ranging from  $5.15\pm0.03 - 9.54\pm0.06$  mg/kg in gills, while liver, muscle and kidney recorded a mean (mg/kg) range of  $2.11\pm0.01-3.91\pm0.03$ ,  $0.88\pm0.01-1.62\pm0.01$  and  $1.86\pm0.01-3.44\pm0.02$  respectively.

### Total Petroleum Hydrocarbon (TPH)

TPH from this study showed that the gills had a mean of 178.82mg/kg which had the highest concentration followed by the mean concentration of 73.32mg/kg in the liver, 64.37mg/kg in Kidney and the least mean concentration of 30.40 mg/kg was recorded in the muscle.

Table 1: Mean±SE of TPH and its aliphatic components in the sampled organs of *Trachurus trecae* in August

Month	Parameters	Mean±SE						
		Liver	Gill	Muscle	Kidney	Min	Max	P-Value
August	Nonane (C 9)	$9.09 \pm 0.06$	22.17±0.15	$3.77 \pm 0.03$	$7.98 \pm 0.05$	3.72	22.32	P<0.001
	Decane(C 10)	$18.54 \pm 0.12$	45.23±0.30	$7.69 \pm 0.05$	$16.28 \pm 0.11$	7.74	45.54	P<0.001
	Dodecane(C 12)	$23.06 \pm 0.15$	56.23±0.37	$9.56 \pm 0.06$	$20.24 \pm 0.13$	9.43	56.62	P<0.001
	Tetradecane(C 14)	$14.69 \pm .10$	35.83±0.24	$6.09 \pm 0.04$	$12.90 \pm 0.09$	6.01	36.08	P<0.001
	Hexadecane(C 16)	$1.31 \pm 0.01$	$3.19 \pm 0.02$	$0.54 \pm 0.004$	$1.15 \pm 0.01$	0.53	3.21	P<0.001
	Octadecane(C 18)	$4.29 \pm 0.03$	$10.47 \pm 0.07$	$1.78 \pm 0.01$	$3.77 \pm 0.03$	1.76	10.54	P<0.001
	Nonadecane(C 19)	$5.16 \pm 0.03$	$12.59 \pm 0.08$	$2.14 \pm 0.01$	4.53±0.03	2.11	12.68	P<0.001
	Eicosane(C 20)	$7.50 \pm 0.05$	$18.28 \pm 0.12$	$3.11 \pm 0.02$	$6.58 \pm 0.04$	3.07	18.41	P<0.001
	Docasane(C 22)	$4.78 \pm 0.03$	$11.65 \pm 0.08$	$1.98 \pm 0.01$	4.19±0.03	1.95	11.73	P<0.001
	Tetracosane(C 24)	$2.35 \pm 0.02$	$5.74 \pm 0.04$	$0.98 \pm 0.01$	$2.07 \pm 0.01$	0.96	5.78	P<0.001
	hexacosane(C 26)	$0.71 \pm 0.01$	$1.74 \pm 0.01$	$0.30 \pm 0.002$	$0.63 \pm 0.004$	0.29	1.75	P<0.001
	Tricosane(C 30)	$1.07 \pm 0.01$	$2.61 \pm 0.02$	$0.44 \pm 0.003$	$0.94 \pm 0.01$	0.44	2.63	P<0.001
	Total Aliphatic Hydrocarbon (mg/kg)	92.56±0.62	225.74±1.50	38.38±0.26	$81.27 \pm 0.54$	37.87	227.29	P<0.001
	$\Sigma$ Poly Aromatic Hydrocarbon							
	(mg/kg)	$3.91 \pm 0.03$	$9.54 \pm 0.06$	$1.62 \pm 0.01$	$3.44 \pm 0.02$	1.62	9.65	P<0.001
	Total Petroleum Hydrocarbon							
	(mg/kg)	96.47±0.59	235.29±1.45	$40.00 \pm 0.25$	$84.70 \pm 0.52$	39.51	236.75	P<0.001

P<0.001 - Highly significant

EU recommended limit of 2µg/kg; wet weight for fish

Table2: Mean±SE of TPH and its aliphatic components in the sampled organs of *Trachurus trecae* in September

Month	Parameters	Mean±SE						
Month		Liver	Gill	Muscle	Kidney	Min	Max	P-Value
	Nonane (C 9)	6.73±0.05	$16.40 \pm 0.11$	$2.79 \pm 0.02$	$5.91 \pm 0.04$	2.75	16.52	P<0.001
	Decane(C 10)	$13.72 \pm 0.09$	33.47±0.22	$5.69 \pm 0.04$	$12.05 \pm 0.08$	5.61	33.70	P<0.001
	Dodecane(C 12)	$17.06 \pm 0.11$	$41.61 \pm 0.28$	$7.07 \pm 0.05$	$14.98 \pm 0.10$	6.98	41.90	P<0.001
	Tetradecane(C 14)	$10.87 \pm 0.07$	$26.52 \pm 0.18$	4.51±0.03	$9.55 \pm 0.06$	4.45	26.70	P<0.001
	Hexadecane(C 16)	$0.97 \pm 0.01$	$2.36 \pm 0.02$	$0.40 \pm 0.003$	$0.85 \pm 0.01$	0.40	2.38	P<0.001
	Octadecane(C 18)	$3.18 \pm 0.02$	$7.75 \pm 0.05$	$1.32 \pm 0.01$	$2.79 \pm 0.02$	1.30	7.80	P<0.001
	Nonadecane(C 19)	3.82±0.03	9.32±0.06	$1.58 \pm 0.01$	$3.35 \pm 0.02$	1.56	9.38	P<0.001
	Eicosane(C 20)	$5.55 \pm 0.04$	$13.53 \pm 0.09$	$2.30 \pm 0.02$	$4.87 \pm 0.03$	2.27	13.62	P<0.001
September	Docasane(C 22)	$3.53 \pm 0.02$	8.62±0.06	$1.47 \pm 0.01$	$3.10 \pm 0.02$	1.45	8.68	P<0.001
-	Tetracosane(C 24)	$1.74 \pm 0.01$	4.25±0.03	$0.72 \pm 0.01$	$1.53 \pm 0.01$	0.73	4.28	P<0.001
	hexacosane(C 26)	$0.53 \pm 0.004$	1.29±0.01	$0.22 \pm 0.002$	$0.46 \pm 0.003$	0.22	1.30	P<0.001
	Tricosane(C 30)	$0.79 \pm 0.01$	$1.93 \pm 0.01$	$0.33 \pm 0.002$	$0.70 \pm 0.01$	0.32	1.95	P<0.001
	Total Aliphatic Hydrocarbon (mg/kg)	68.49±0.46	167.05±1.11	$28.40 \pm 0.19$	$60.14 \pm 0.40$	28.02	168.19	P<0.001
	ΣPoly Aromatic Hydrocarbon							
	(mg/kg)	$2.90 \pm 0.02$	$7.06 \pm 0.05$	$1.20 \pm 0.01$	$2.54 \pm 0.02$	1.19	7.14	P<0.001
	Total Petroleum Hydrocarbon							
	(mg/kg)	$71.39 \pm 0.44$	174.11±1.07	$29.60 \pm 0.18$	62.68±0.39	29.24	175.19	P<0.001

P<0.001- Highly significant

EU recommended limit of 2µg/kg; wet weight for fish

Table 3: Mean±SE of TPH and its aliphatic components in the sampled organs of *Trachurus trecae* in October

Month	Parameters	Mean±SE						
		Liver	Gill	Muscle	Kidney	Min	Max	P-Value
	Nonane (C 9)	4.91±0.03	$11.97 \pm 0.08$	$2.04 \pm 0.01$	4.31±0.03	2.01	12.05	P<0.001
	Decane(C 10)	$10.01 \pm 0.07$	$24.42 \pm 0.16$	4.15±0.03	$8.79 \pm 0.06$	4.10	24.59	P<0.001
	Dodecane(C 12)	$12.45 \pm 0.08$	$30.37 \pm 0.20$	$5.16 \pm 0.03$	$10.93 \pm 0.07$	5.09	30.57	P<0.002
	Tetradecane(C 14)	$7.93 \pm 0.05$	$19.35 \pm 0.13$	$3.29 \pm 0.02$	$6.97 \pm 0.05$	3.25	19.48	P<0.00
	Hexadecane(C 16)	$0.71 \pm 0.01$	$1.72 \pm 0.01$	$0.29 \pm 0.002$	$0.62 \pm 0.004$	0.29	1.73	P<0.00
	Octadecane(C 18)	$2.32 \pm 0.02$	$5.65 \pm 0.04$	$0.96 \pm 0.01$	$2.04 \pm 0.01$	0.95	5.69	P<0.00
	Nonadecane(C 19)	$2.79 \pm 0.02$	$6.80 \pm 0.05$	$1.16 \pm 0.01$	$2.45 \pm 0.02$	1.14	6.85	P<0.00
	Eicosane(C 20)	$4.05 \pm 0.03$	$9.87 \pm 0.07$	$1.68 \pm 0.01$	$3.55 \pm 0.02$	1.66	9.94	P<0.00
October	Docasane(C 22)	$2.58 \pm 0.02$	$6.29 \pm 0.04$	$1.07 \pm 0.01$	$2.26 \pm 0.02$	1.06	6.33	P<0.00
	Tetracosane(C 24)	$1.27 \pm 0.01$	$3.10 \pm 0.02$	$0.53 \pm 0.04$	$1.12 \pm 0.01$	0.52	3.12	P<0.00
	hexacosane(C 26)	$0.38 \pm 0.003$	$0.94 \pm 0.01$	$0.16 \pm 0.001$	$0.34 \pm 0.002$	0.16	0.95	P<0.00
	Tricosane(C 30)	$0.58 \pm 0.004$	$1.41 \pm 0.01$	$0.24 \pm 0.002$	$0.51 \pm 0.003$	0.24	1.42	P<0.00
	Total Aliphatic Hydrocarbon (mg/kg)	$47.98 \pm 0.33$	$121.90 \pm 0.81$	$20.72 \pm 0.14$	43.88±0.29	20.45	20.45	P<0.00
	$\Sigma$ Poly Aromatic Hydrocarbon							
	(mg/kg)	$2.11 \pm 0.01$	$5.15 \pm 0.03$	$0.88 \pm 0.01$	$1.86 \pm 0.01$	0.87	5.21	P<0.00
	Total Petroleum Hydrocarbon							
	(mg/kg)	$52.09 \pm 0.32$	$127.06 \pm 0.78$	$21.60 \pm 0.13$	$45.74 \pm 0.28$	21.33	127.84	P<0.00

P<0.001- Highly significant

EU recommended limit of 2µg/kg; wet weight for fish

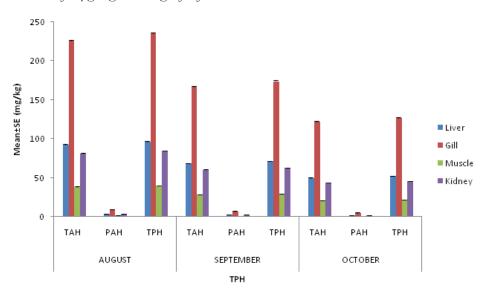


Figure 1: Mean±SE of TPH in organs of Trachurus trecae sampled from August to October

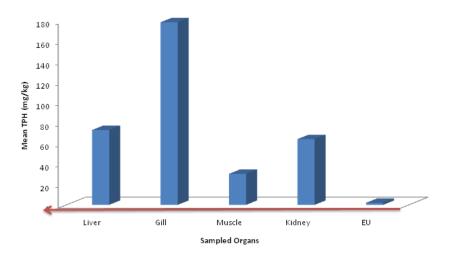


Figure 2: Mean TPH analysed in the organs of Trachurus trecae compared to EU standard

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## DISCUSSION

TPH was found in all the fish samples with varying concentrations. All fish ingest petroleum hydrocarbons directly or indirectly from contaminated water, in food and sediments leading to massive destruction of aquatic biota (Asuquo and Ewa-Oboho 2004). Both of the aliphatic and polycyclic aromatic hydrocarbon fractions of dissolved petroleum are readily absorbed by most finfish and shellfish because of their high lipid solubility and are bioconcentrated in them (Gobas *et. al.*, 1999). This study analyzed these components in the liver, gills, muscle and kidney of *Trachurus trecae* for a three-months period (August–October 2014).

Twelve components of n-alkanes making up the TAH were analyzed. Across the sampled period, the gills had the highest mean concentrations of TAH while the muscles had the lowest concentration. There was a high level of significance when the values were analyzed across the various organs. The high concentration of TAH in the gills could be due to the constant interaction of the gills which are highly vascularized with the source of pollution. Respiration in particular keeps this region constantly exposed to contaminants in the water. The low content of TAH in the muscles suggests that bioaccumulation is lower as against uptake demonstrated by the gills. TAH from this study was higher than what was reported by Olaji et al., (2014) except Docosane which had a mean±SE value of 1123.70±952.10µg/kg.

In this study, PAH is reported as  $\Sigma$ PAH. The values reported followed similar trend as observed for TAH. The gills had the highest mean concentrations while the muscles had the lowest concentration. There was a high level of significance when the values were analyzed across the various organs. According to GESAMP (1982), fish tend to concentrate PAHs in their tissues when exposed to petroleum, but they do not retain it indefinitely, leading to these compounds not accumulating in very high concentration in edible tissues. The accumulation and depuration of PAHs in fish can be influenced by various factors including route and duration of exposure, lipid content of tissues, environmental

factors, differences in species, age, and sex, and exposure to other xenobiotics (Varanasi *et al.*, 1987). The gills recorded the highest mean concentration while the least concentrations were reported in the fish muscles. The PAH from this study is higher than the values reported by Olaji *et al.* 2014 when TPH was investigated in four species of fish at Degele Community, Nigeria.

The highest TPH was recorded in the month of August and the gills had TPH as high as 235.29mg/kg and as low as 40.00mg/kg in muscles. In August, liver and kidney had a mean value of 96.47 and 84.70mg/kg respectively. The mean concentrations for the entire study are 73.31, 178.82, 30.40 and 64.37mg/kg in liver, gills, muscles and kidney respectively. The high values reported in the kidney are similar to the fact that it has a direct relationship with the contaminated medium. Relatively high values in the kidney and liver suggest centres of elimination and biotransformation/detoxification respectively. The muscles are the storage point for these contaminants and the concentration suggest the net from uptake, elimination, detoxification and depuration. The high concentrations observed in comparison with EU standards (2µg/kg) also suggest their ability to accumulate hydrocarbon in their tissue (Ansari et al., 2012). High concentration of hydrocarbons in these species is probably due to the higher lipid content of their muscle tissue (Shriadah 2001). This result agrees with the findings of Ashraf and Mian (2008) that TPH concentration not only varies between the tissues of different fish species but it also varied in the same species depending on the season. However, the TPH in the muscles from this study was higher than the highest level of TPH 7.4±3.2 µg-g-1 reported for Scarus ghabon (Ashraf and Mian, 2008).

The results from this study are higher than that reported by Tolosa *et al.* (2005) who reported TPH concentration in tissues of *Epinephelus coioides* (2.07 $\mu$ g-g-1) and *Lethrinus nebulosus* (3.40  $\mu$ g-g-1) caught from Al Marfa, UAE. In addition, The range of values observed in the present study are higher than the reported concentration range of 0.52 to 2.05  $\mu$ g g-1 from Tamilnadu coast (Veerasingam *et al.*, 2011) and those of 0.47 – 3.77  $\mu$ g g-1 (Av. 3.67  $\mu$ g g-1) reported in the fish tissue

of North and Central Arabian Sea (Gupta *et al.*,1993). In a more recent study Chouksey *et al.* (2004) reported PHC residue between 1.8 and 10.8 ppm (wet wt) in five fish species off Mumbai coast. However, the levels were above EU recommended limit of  $2\mu g/kg$  for fish.

Both of the aliphatic and polycyclic aromatic hydrocarbon fractions of dissolved petroleum are readily absorbed by most finfish and shellfish because of their high lipid solubility and are bioconcentrated in them (Gobas et al., 1999). In the long term, the sub-lethal dose of oil and oil products have produced physiological and histopathological changes that can affect marine life by interfering with the ability to breed, reproduce, grow, or perform other vital functions. In extreme cases death and development of genetic mutation in fish and shellfish may occur (Anderson et al., 1974). Recently, studies have shown that most human cancers such as prostrate and lung cancer can be attributed to dietary sources (Dhananjayan et al., 2012; Shen et al., 2008).

## CONCLUSION

The present study gives the baseline distribution of total petroleum hydrocarbon in fish tissue of Trachurus trecae imported into Nigeria and consumed in Benin City. The investigation revealed that all the fish samples analyzed had TPH concentrations in their organs (liver, gills, muscle, and kidney). The concentration showed a trend gills > liver > kidney > muscle. The mean concentration in the fish muscle studied (mostly consumed by humans), though was low compared to other analyzed organs, but were greater than EU recommended benchmark of  $2\mu g/kg$  for fish. The high concentration of TPH in the tissue from this investigation suggests that the muscle is a high bioaccumulator. Since the muscle is the main source of protein for food, it can pose health risk to man. Periodical monitoring of fish imports for TPH levels is suggested for public health protection.

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