Polycyclic Aromatic Hydrocarbons (PAH) in fingerlings of *Clarias gariepinus* (Burchell, 1822) to Petroleum

Ezike Christopher Onyemaechi [1]*and Echor Felix Okaliwe [1]

[1]Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology
*Correspondence: Christopher.ezike@esut.edu.ng

Abstract
One hundred and twenty (120) fingerlings of *Clarias gariepinus* (mean weight: 0.96 ± 0.1g) were randomly exposed to 4 experimental treatments of petroleum, based on LC_{50} values (6.4mg/L of crude oil, 8.7mg/L of petrol, 8.0mg/L of kerosene and 7.8mg/L of diesel oil) and replicated thrice, to determine polycyclic aromatic hydrocarbons (PAH) in exposed fish for 96 h. There was no significant difference ($P > 0.05$) in total (PAHs) between crude oil (97.1 ng/uL) and diesel (97.2 ng/uL) exposed fish and also between petrol (53.2 ng/uL) and kerosene (49.6 ng/uL) exposed fish, but there was a significant difference ($P < 0.05$) in PAH levels of the crude oil/diesel exposed -groups of fish compared to petrol/kerosene exposed -groups of fish (97.1/97.2 and 53.2/49.6 ng/uL). Naphthalene correlated positively to benzo a anthracene (r=0.672, ($P < 0.05$), benzo b fluoranthene (r=0.681, $P < 0.05$) and chrysene (r=0.615, $P < 0.05$) but did not correlate to fluorene. Benzo a anthracene correlated positively to benzo a pyrene (r=0.578, $P < 0.05$), phenathrene (r=0.685, $P < 0.05$) but did not correlate to acenaphthene. Fluorene correlated positively to benzo a pyrene (r=0.695, $P < 0.05$) but did not correlate to chrysene. Chrysene correlated positively to dibenzo a,h, pyrene (r=0.658, $P < 0.05$) to phenathrene and benzo b fluoranthene (r=0.659, $P< 0.05$). Indeno 123 cd- pyrene and fluranthene however did not correlate to other PAHs except naphthanene, acenaphthene and acenaphthylene. The level of PAH in fish may translate to the toxicity effect since crude oil and diesel with lower LC_{50} (6.4 and 7.8 mg/L) deposited greater PAH than kerosene and petrol with higher LC_{50} (8.7 and 8.0 mg/L) in fingerlings of *C. gariepinus*. High risk to cancer disorders may occur in exposed fish to petroleum with high incidence of fluorene , anthracene, pyrene and benz a anthracene which correlated positively to benzo a pyrene which provide some basis for predicting impact of oil spills on fingerling population.

Key words: polycyclic aromatic hydrocarbons; petroleum; *Clarias gariepinus*

1. **Introduction**

Polycyclic Aromatic Hydrocarbons (PAHs) are group of compounds consisting of two or more fused aromatic rings which are formed during incomplete combustion of organic materials such as wood and fossil fuels, petroleum products and coal (Li *et al.,* 2001). They are ubiquitous pollutants frequently found in a variety of environments such as freshater marine sediments, atmosphere and ice [3]. Many PAHs and their expoxides are highly toxic to microorganisms, fishes and man [15]. In recent times, PAHs have received much attention due to their potential cause of cancer, mutagenic diorders and birth defects [15, 11]. Adverse effects of PAHs have also been observed in marine organisms and they include growth reduction, endocrine alteration, and malformation of embryo and larvae and DNA damage. Ingestion of contaminated food and diffusion from water across their gills and skin are major routes of PAHs exposure to fish. Due to the lipophilic nature and high chemical stability of PAHs, they accumulate in the fatty tissues of fish following their uptake. Fishes are therefore good indicators of pollution in inland and coastal waters [5]. Two broad groups exist based on their physical and biological properties including, high molecular weight (HMW) and low molecular weight (LMW) PAHs. The HMW PAHs consists of 4-6 aromatic rings and are less readily bio-degraded by indigenous microorganisms, hence can persist in the aqueous environment by bio-accumulating in aquatic organisms like fish and mussels and are more carcinogenic. The LMW PAHs consists of 2-3 aromatic rings and although less carcinogenic, also pose toxic effect to many aquatic organisms [2]. The concentrations of petroleum products toxic to aquatic organisms depend on the type and hydrocarbon constituents, as well as the species involved. [10] Estimated concentrations of petroleum toxic to fish eggs and fingerlings to be 0.5-10 mg/L. Benzo a pyrene binds to DNA to cause cancer and is frequently used as marker for carcinogenic disorders and may provide basis for predicting impact of exposures of PAH to *C.gariepinus* fingerlings[14].

Although fishes have oxidative enzymes for metabolic detoxification of xenobiotics including aromatic petroleum hydrocarbons [14], little is known about the PAH level of petroleum in exposed fish. The uptake and translocation of crude oil and other petroleum products /or compounds in fish may be the gills, guts or the intestinal wall [17]. The parent compounds readily solublize in cell membranes and are probably carried via the erythrocytes to the general circulation of the blood. Some of the compounds may be carried by lipoproteins and leukocytes in the blood to the liver. The major route

of excretion of petroleum metabolites is through the bile; into the intestine and out with faeces. Some are excreted through the gills and kidney [7].
The African catfish of genus *Clarias* are esteemed group of fish with high market value in Tropical Africa [13]. Their hardy nature and possession of accessory air-breathing organs enable them to tolerate adverse aquatic conditions [4]. Nonetheless, *Clarias gariepinus* fingerlings are very delicate and sensitive to aquatic pollutants including crude oil and other petroleum products[16]. This study was undertaken to determine the comparative toxic level of PAH of various petroleum products on *C. gariepinus* fingerlings

2. Results and Discussion

No significant difference ($P > 0.05$) in total (PAHs) between crude oil (97.1 ng/uL, Table 1) and diesel (97.2 ng/uL) exposed fish and between petrol (53.2 ng/uL) and kerosene (49.6 ng/uL) exposed fish gave an indication that kerosene and petrol PAH in fish exposed for 96h are similar as well as PAH in fish to crude oil and diesel for the same period of time. A significant difference ($P < 0.05$) in PAH levels of the crude oil/diesel exposed -groups of fish compared to petrol/kerosene exposed -groups of fish (97.1/97.2 and 53.2/49.6 ng/uL, Table 1) shows that heavier petroleum deposit more PAH in fish than light ones after short exposure duration for 4 days [6]. Naphthalene correlated positively to benz a anthracene (r=0.672, ($P < 0.05$, Table 2), benzo b fluoranthene (r=0.681, $P < 0.05$) and chrysene (r=0.615, $P < 0.05$) but did not correlate to fluorene. Benz a anthracene correlated positively to benzo a pyrene (r=0.578, $P < 0.05$), phenathrene (r=0.685, $P < 0.05$) but did not correlate to acenaphthene. Fluorene correlated positively to benzo a pyrene (r=0.695, $P < 0.05$) but did not correlate to chrysene. Chrysene correlated positively to dibenz a,h, pyrene (r=0.658, $P < 0.05$) to phenathrene and benzo b fluoranthene (r=0.659, $P< 0.05$). Indeno 123-cd pyrene and fluoranthene however did not correlate to other PAHs except naphthanene, acenaphthene and acenaphthylene. The lighter PAH LMW were more predominant in kerosene and petrol (naphthalene, acenaphthene and acenaphthylene) in exposed fish, and correlated more than heavier PAH HMW (indeno 123 cd pyrene and fluoranthene). The level of PAH in fish may translate to toxicity effect since crude oil and diesel with lower LC_{50} (6.4 and 7.8 mg/L) and therefore more toxic [6], deposited greater PAH than kerosene and petrol with higher LC_{50} (8.7 and 8.0 mg/L) and therefore less toxic to fingerlings of *C. gariepinus*. Greater PAH in HMW than LMW agrees with the findings of [2] and may provide basis for predicting the impact of oil spills on fingerlings population. High risk to cancer disorders may occur in exposed fish to petroleum with elevated levels of fluorene , anthracene, pyrene and benzo a anthracene which correlated positively to benzo a pyrene (Figure 1) frequently used as carcinogenic marker [14].

Table 1: PAH in fish exposed to Petroleum

Petroleum	Number of PAH	Total PAH (ng/uL)
Petrol (8.0 mg/L)	15	53.25±5.33
Kerosene (8.7 mg/L)	15	49.64±4.90
Diesel (7,8 mg/L)	15	97.25±6.23
Crude (6.4mg/L)	15	97.17±6.32

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 January 2018 doi:10.20944/preprints201801.0009.v1

Table 2: Pearson Correlation of PAH

		Naphthalene	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene	Pyrene	Benz a anthracene	Chrysene	Benzo ghi fluoranthene	Benzo a pyrene	Dibenz ah anthracene	Benzo ghi pyrene	Indeno 123-cd pyrene	Fluoranthene
Naphthalene	Pearson Correlation	1	.965**	.753**	.563	.743**	.827**	.828**	.672*	.615*	.681*	.403	.943**	.881**	.777**	.824**
	Sig. (2-tailed)		.000	.005	.057	.006	.001	.001	.017	.033	.015	.194	.000	.000	.003	.001
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Acenaphthylene	Pearson Correlation	.965**	1	.820**	.534	.685*	.817**	.814**	.656*	.674*	.703*	.426	.983**	.900**	.838**	.882**
	Sig. (2-tailed)	.000		.001	.074	.014	.001	.001	.020	.016	.011	.167	.000	.000	.001	.000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Acenaphthene	Pearson Correlation	.753**	.820**	1	-.013	.225	.357	.361	.258	.966**	.354	.014	.862**	.571	.985**	.985**
	Sig. (2-tailed)	.005	.001		.968	.483	.255	.249	.418	.000	.258	.965	.000	.053	.000	.000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Fluorene	Pearson Correlation	.563	.534	-.013	1	.905**	.907**	.912**	.828**	-.235	.796**	.695*	.478	.796**	.007	.094
	Sig. (2-tailed)	.057	.074	.968		.000	.000	.000	.001	.462	.002	.012	.116	.002	.982	.771
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Phenanthrene	Pearson Correlation	.743**	.685*	.225	.905**	1	.902**	.922**	.862**	.012	.773**	.759**	.658*	.850**	.257	.331
	Sig. (2-tailed)	.006	.014	.483	.000		.000	.000	.000	.969	.003	.004	.020	.000	.421	.294
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Anthracene	Pearson Correlation	.827**	.817**	.357	.907**	.902**	1	.991**	.815**	.156	.867**	.641*	.760**	.925**	.383	.462
	Sig. (2-tailed)	.001	.001	.255	.000	.000		.000	.001	.629	.000	.025	.004	.000	.219	.130
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Pyrene	Pearson Correlation	.828**	.814**	.361	.912**	.922**	.991**	1	.863**	.157	.866**	.652*	.768**	.939**	.377	.457
	Sig. (2-tailed)	.001	.001	.249	.000	.000	.000		.000	.627	.000	.022	.004	.000	.227	.135
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Benz a anthracene	Pearson Correlation	.672*	.656*	.258	.828**	.862**	.815**	.863**	1	.060	.791**	.578*	.618*	.869**	.254	.318
	Sig. (2-tailed)	.017	.020	.418	.001	.000	.001	.000		.853	.002	.049	.032	.000	.425	.314
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Chrysene	Pearson Correlation	.615*	.674*	.966**	-.235	.012	.156	.157	.060	1	.205	-.171	.716**	.380	.947**	.924**
	Sig. (2-tailed)	.033	.016	.000	.462	.969	.629	.627	.853		.522	.596	.009	.224	.000	.000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Benzo b fluoranthene	Pearson Correlation	.681*	.703*	.354	.796**	.773**	.867**	.866**	.791**	.205	1	.516	.659*	.887**	.348	.401
	Sig. (2-tailed)	.015	.011	.258	.002	.003	.000	.000	.002	.522		.086	.020	.000	.268	.196
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Benzo a pyrene	Pearson Correlation	.403	.426	.014	.695*	.759**	.641*	.652*	.578*	-.171	.516	1	.415	.535	.099	.105
	Sig. (2-tailed)	.194	.167	.965	.012	.004	.025	.022	.049	.596	.086		.180	.073	.759	.746
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Dibenz ah anthracene	Pearson Correlation	.943**	.983**	.862**	.478	.658*	.760**	.768**	.618*	.716**	.659*	.415	1	.870**	.867**	.909**
	Sig. (2-tailed)	.000	.000	.000	.116	.020	.004	.004	.032	.009	.020	.180		.000	.000	.000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Benzo ghi pyrene	Pearson Correlation	.881**	.900**	.571	.796**	.850**	.925**	.939**	.869**	.380	.887**	.535	.870**	1	.575	.636*
	Sig. (2-tailed)	.000	.000	.053	.002	.000	.000	.000	.000	.224	.000	.073	.000		.051	.026
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Indeno 123-cd pyrene	Pearson Correlation	.777**	.838**	.985**	.007	.257	.383	.377	.254	.947**	.348	.099	.867**	.575	1	.983**
	Sig. (2-tailed)	.003	.001	.000	.982	.421	.219	.227	.425	.000	.268	.759	.000	.051		.000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Fluoranthene	Pearson Correlation	.824**	.882**	.985**	.094	.331	.462	.457	.318	.924**	.401	.105	.909**	.636*	.983**	1
	Sig. (2-tailed)	.001	.000	.000	.771	.294	.130	.135	.314	.000	.196	.746	.000	.026	.000	
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

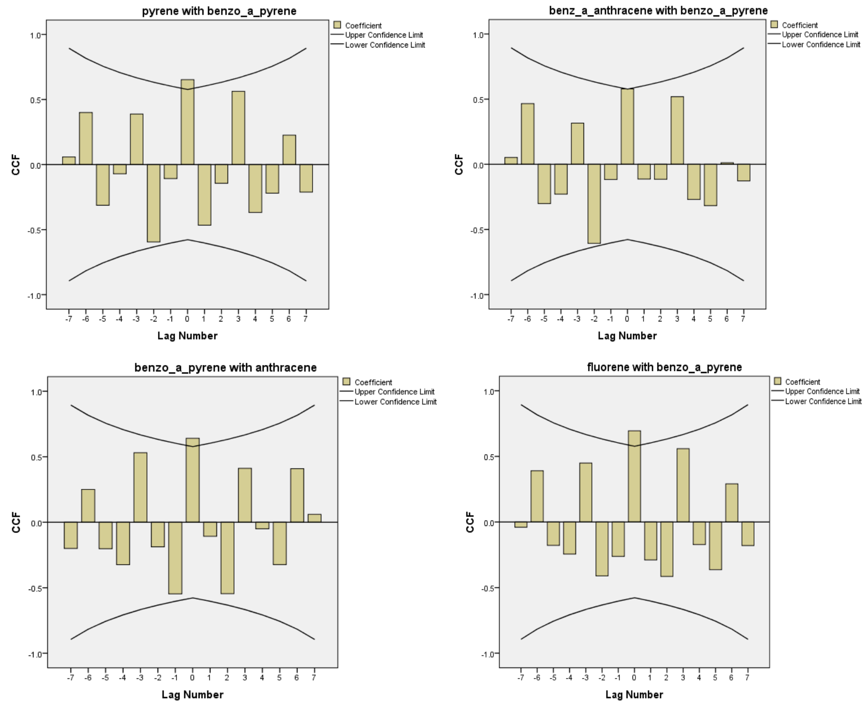

Figure 1: Positive correlation of benzo a pyrene with pyrene, anthracene, benz a anthracene and pyrene

2.1 Experimental fish and petroleum

A total of one hundred and twenty (120) juveniles of African catfish (mean weight 0.96 ± 0.1g) were obtained from local outskirts in Enugu Nigeria and transported to Fisheries Wet Laboratory of the Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology ESUT, Enugu Nigeria. They were held in four fiber reinforced plastic (FRP) tanks, containing 320 L of de-chlorinated tap water. Aeration was provided to all tanks round the clock in order to maintain dissolved oxygen contents. Before the commencement of the study, the fish were acclimatized for two weeks and were fed with commercial fish diet composed of 40% crude protein. The faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Petroleum was obtained from Nigerian National Petroleum Cooperation Enugu. Ethical clearance from the Enugu State University of Science and Technology Committee on Experimental Animal Care was obtained and followed.

2.2 Acute toxicity test

Toxicity of petroleum to *C. gariepinus* was carried out according to the OECD guideline for testing of chemicals No. 203 in a semi-static renewal system by using 200L capacity glass aquaria. Thirty (30) fish were randomly exposed to 4 experimental treatments of petroleum based on LC_{50} values (6.4 mg/L of crude oil, 8.7 mg/L of petrol, 8.0mg/L of kerosene and 7.8mg/L of diesel oil) [10,13] and replicated thrice, to determine polycyclic aromatic hydrocarbons (PAH) in exposed fish for 96 h. The exposure pollutant was renewed each day and was also analysed using LC–MS/MS to ensure the agreement between nominal and actual concentrations of the petroleum in the aquaria [9]. The experiment was conducted under the natural photoperiod of 12:12 light-dark cycle. The physico-chemical parameters of the test water were analysed daily, using standard methods [1] and were recorded (dissolved oxygen 7.50 ± 0.45 mg L^{-1} , temperature 27.75 ± 0.5 ^{0}C, pH 7.8 ± 0.13 and free carbon dioxide 4.28 ± 0.6 mg L^{-1}) [13]. The test fish were sampled in each replicate to determine level of PAH in exposed fish. A portion of each sample was taken for extraction and analysis of PAH [12].

2.3 Statistical Analysis

Data obtained were expressed as standard mean ± standard error of mean and analyzed using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago Illinois, USA). Differences in the test between low molecular weight LMW and high molecular weight HMW PAHs were subjected to one way analysis of variance (ANOVA) followed by Turkey's multiple range test to determine level of difference at 95% probability level. Pearson correlation was used to determine relationship between individual PAHs.

3. Conclusion

The level of PAH in fish may translate to toxicity effect since crude oil and diesel with lower LC_{50} (6.4 and 7.8 mg/L) deposited greater PAH than kerosene and petrol with higher LC_{50} (8.7 and 8.0 mg/L) in fingerlings of *Clarias gariepinus*. The quality and quantity of PAH in exposed fish to petroleum may provide some basis for predicting the impact of oil spills on fingerling population. High risk to cancer disorders may occur in exposed fish to petroleum with incidence of fluorene , anthracene, pyrene and benz a anthracene which correlated to benzo a pyrene.

References

1. APHA. American Public Health Association, American Water works Association and Water Environmental Federation). Standard Methods of Examination of water and Wastewater. 21st ed. APHA 2005, Washington DC, pp 20001-23710.
2. Brown, J.; Peake, B. Sources of heavy metals and polycyclic aromatic hydrocarbons in urban storm water runoff. *Sci. Total Environ.* 2006:359:145–155.
3. Choi, H.; Harrison, R.; Komulainen, H.; Delgado, S. J. Polycyclic aromatic hydrocarbons.WHO Guidelines for Indoor Air Quality: Selected Pollutants. Geneva: World Health Organization 2010. Retrieved 2014-12-12.
4. Hylland, K. Polycyclic aromatic hydrocarbon (PAH) ecotoxicology in marine systems. *Journal of Toxicology and Environmental Health* 2006*,* Part A 69 (1-2): 109-123.
5. Ezike, C.O.; Echor, F.O.; Malachy, N.O.A.; Vera, L.M. Butrylacetycholinesterase activites in liver and plasma, liver glycogen and plasma glucose content, haematology and behaviour of Clariid Catfish Clarias gariepinus to Dichlorvos International Journal of Advanced Fisheries and Aquatic Sciences, 2017 3(1): 90-105. Doi: https://doi.org/10.23953/cloud/ijafas.332. crossref:23953/cloud.ijafas.332
6. Lawal, A.T. Polycyclic aromatic hydrocarbons a review. *Environmental Science* 2017, 3: 1339841. https://doi.org/10.1080/23311843.2017.1339841.
7. Lee, R. F. Metabolism of petroleum hydrocarbons in marine sediments. In: Sources, effects and sinks of hydrocarbons in aquatic environment. *American Institute Biological Sciences* 1976, 333-344.
8. Li, N.; Leu, H. K. Solid phase extraction of polycyclic hydrocarbons in surface water. *J. Chromatogr.* 2001, A, 921:255-263.
9. Li, Z.H.; Velisek, J.; Zlabek, V. Chronic toxicity of verapamil on juvenile rainbow trout (Oncorhynchus mykiss): effects on mor-phological indices, hematological parameters and antioxidant responses. *J. Hazard Mat* 2011, 185:870–880.
10. Lonning, S. The effects of crude oil and oil products on marine fish larvae. Astarte 1977, 10: 37-47. ISSN 0044-7447. Retrieved 2015-04-09.
11. Martinez, E.; Gros, M.; Lacorte, S.; Barcel, D. Simplified procedures for the analysis of polycyclic aromatic hydrocarbons in water sediments and mussels. *J. Chromatogr* 2004, A, 1047:18-188.
12. Neff, J. M. Polycyclic aromatic hydrocarbons. Pages 416-454 in: Rand G.M. and Petrocelli S.R. (eds.). Fundamentals of aquatic toxicology. Hemisphere Publ. Corp. 1985, New York.
13. Nwamba, H. O.; Ugwu, L. L. C.; Aghamuo, H. U. Effect of Nigerian Bonny light crude oil on mortality rate of Heterobranchus bidorsalis fingerlings. In: *Proceedings of 16th Annual Conference and International Workshop of the Nigerian Association of Aquatic Science* 3rd – 6th October 2001.
14. Payne, J. F.; Penrose, W.R. Induction of akyl hydrocarbon (benzo (a) Pyrene) hydroxylase in fish by petroleum. *Bull Environ. Contam. Toxicol.* 1975, 14: 112-226.
15. Ramesh, A.; Archibong, A.; Hood, D. B.; Guo, Z.; Loganathan, B. G. Global environmental distribution and human health effects of polycyclic aromatic hydrocarbons. Global Contamination Trends of Persistent Organic Chemicals. Boca Raton 2011, FL: CRC Press. Pp. 97-126.
16. Reed, W. J. Burchard, Hopson, A.J., Jonathan, J. and Ibraham, Y. (1967). Fish and fisheries of Northern Nigeria. Govt. Press, London, 226pp.
17. Roubal, W.T.; Collier, T.K.; Malins, D.C. Accumulation and metabolism of carbon – 14 labelled benzene, naphthalene and Anthracene by young cohosalmon (Onchorynchus Kisutch) Arch. *Environ. Contam. Toxicol.* 1977*,* 5: 515-529.