E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(5): 62-67

www.fisheriesjournal.com
Received: 26-07-2019
Accepted: 30-08-2019

Christopher Onyemaechi Ezike
Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology, Enugu, Nigeria

Felix Okaliwe Echor
Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology, Enugu, Nigeria

Igugo Raymond
Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology, Enugu, Nigeria.

Nicholas Chinwe Uwadiegwu
Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology, Enugu, Nigeria

Corresponding Author:
Christopher Onyemaechi Ezike
Department of Animal/Fisheries Science and Management, Enugu State University of Science and Technology, Enugu, Nigeria

Benzo [a] Pyrene-carcinogenic and mutagenic equivalents of *Clarias gariepinus* (Burchell, 1822) to Nigerian-crude and diesel oils

Christopher Onyemaechi Ezike, Felix Okaliwe Echor, Igugo Raymond and Nicholas Chinwe Uwadiegwu

Abstract
The toxicity and PAH of *C. gariepinus* (0.9±0.01g) to 1, 3, 6, 9, 12 and 0.0 ml/L of crude oil and diesel was determined. There was no mortality in the control but 10, 20, 50 and 60% occurred in crude oil and 10, 20, 30, 40 and 60% in diesel after 96 h exposure. The logarithmic probit lines and R^2: y = 1.372x + 3.724, R^2 = 0.997 and y = 1.665x + 3.694, R^2 = 0.998 and 96-h LC_{50}: 9.6 and 11.0 ml/L represented toxicity values for crude oil and diesel. Total PAH in exposed fish ranged from the highest value of 99.68±4.81 crude oil > 97.30± 14.57 diesel oil ng/µL the lowest value of 0.061 Nap to the highest value of 384.68 in DahA for crude oil and Diesel oil indicated lowest value of 2.22 Ace to highest value of 389.05 ng/µL DahA. Mean Σ 16PAH of lighter product showed greater toxicity than heavier products require further investigation to the pharmacokinetics of the products on fishes.

Keywords: Toxic equivalent factor, carcinogenicity, mutagen, PAH, petroleum

1. Introduction
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 [11, 10, 6]. 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 [14, 13]. 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 a marker for carcinogenic disorders and may provide the basis for predicting the impact of exposures of PAH to *C. gariepinus* fingerlings [18]. BaP-TEQ (carcinogenic equivalents and BaP- MEQ (mutagenic equivalents are measure for sum of total 8 number of particulate PAHs (Σ8PAH), having molecular weight greater than 228 grams. Σ8PAH includes benzo (a) pyrene (BaP), benz (a) anthracene (BaA), chrysene/iso-chrysene (CHR), benz (b) fluoranthene (BbF), benzo (k) fluoranthene (BkF), indo (123-cd) pyrene (IP), Dibenz (a, h) anthracene (DahA) and benzo (ghi) pyrelene (BghiP) [17, 4, 5]. The hardy nature and possession of accessory air-breathing organs enable C. gariepinus to tolerate adverse aquatic conditions [21]. Nonetheless, *Clarias gariepinus* fingerlings are very delicate and sensitive to aquatic pollutants including crude oil and other petroleum products. This study was undertaken to determine the comparative toxicity and levels of PAH of various Nigerian- petroleum products on *C. gariepinus* fingerlings.

2. Materials and Methods
2.1 Experimental fish and petroleum
A total of seven hundred and twenty (720) fingerlings of African catfish (mean weight 0.96 ± 0.1g) were obtained from local outskirts in Enugu Nigeria and transported to Fisheries

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 (crude oil, petrol, kerosene and diesel) was obtained from Nigerian National Petroleum Cooperation Enugu. The water-soluble fraction WSF was prepared following the method of [25], which involved 20-h mixing of 10:1 clean water to petroleum with a rotator magnetic stirring rod, separated layers after resting for 12-hrs with separating flask before storing as stock solution in corked 50L plastic gallons. Ethical clearance from 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 200 L capacity glass aquaria. Thirty (30) fish per treatment were randomly exposed to 6 experimental treatments (1, 3, 6, 9, 12 and 0 ml/L of water soluble fractions WSF which served as the control of each triplicate group of petroleum product (crude oil and diesel), to determine 96h LC_{50} using the probit analysis proposed by [7, 26] and polycyclic aromatic hydrocarbons (PAH) in exposed fish [14]. The exposure pollutant was renewed each day and was analyzed using LC-MS/MS to ensure the agreement between nominal and actual concentrations of the petroleum in the aquaria. The experiment was conducted under the natural photoperiod of 12:12 light-dark cycle. The physico-chemical parameters of the test water were analyzed 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}). The test fish of 9 and 12 ml/L in each product were sampled to determine Σ 16PAH, Σ 8PAH, TEQ and MEQ of each product. A portion of each sample using the GC-MC was taken for extraction and analysis of PAH [24, 16].

2.3 PAH extraction

The method described by [24, 12, 15] with slight modification for extraction and dosing of PAHs was employed.

The toxic equivalent factors (BaP_{TEF}) and mutagenic equivalent factors (BaP_{MEF}) relating the carcinogenic mutagenic potency of individual PAH to BaP have been used [17, 23, 5]. The BaP carcinogenic equivalent (BaP_{TEF}) and BaP mutagenic equivalent (BaP_{MEQ}) for the individual PAHs was calculated: $BAP_{TEQ} = \Sigma C_1 \text{ X } BAP_{TEF}$; $BAP_{MEQ} = \Sigma C_1 \text{ X } BAP_{MEF}$, where Ci = concentration of PAHs.

2.4 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).

3. Results

3.1 Mortality and Logarithmic probit line for 96-h LC_{50} of petroleum products to *C. gariepinus*

Figure 1 gave mortality values of fish to various concentrations (0, 1, 3, 6, 9 and 12 ml/L. There was no mortality in the control but 10, 20, 50 and 60% for crude oil and 10, 20, 30, 40 and 60% for diesel occurred respectively in 1, 3, 6, 9 and 12 ml/L after 96 hour exposure. Figure 1 gave the logarithmic probit lines and R^2: y = 1.372x + 3.724, R^2 = 0.997 and y = 1.665x + 3.694, R^2 = 0.998 and 96-h LC_{50}: 9.6 and 11.0ml/L for crude oil and diesel to *C. gariepinus*

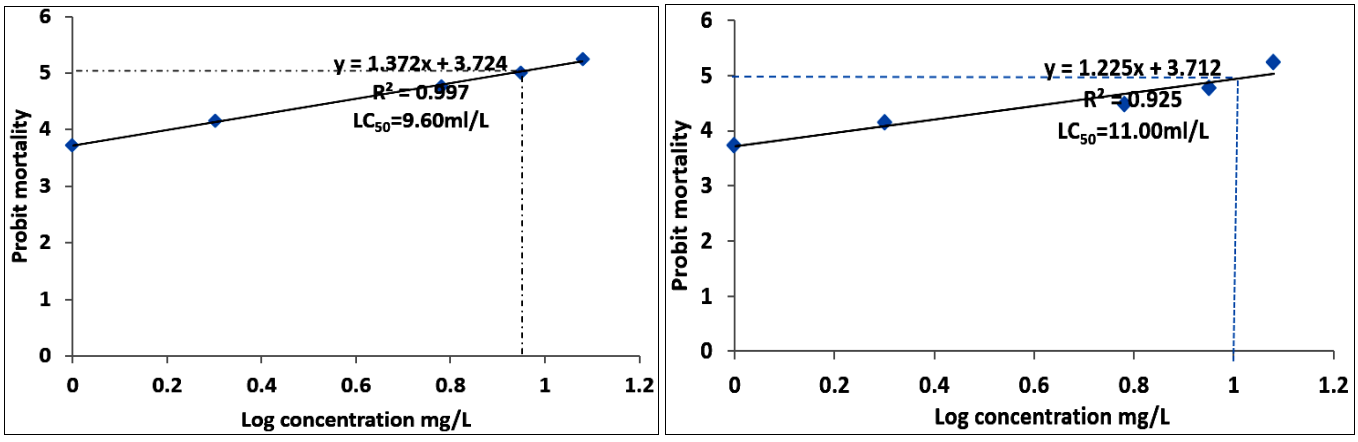
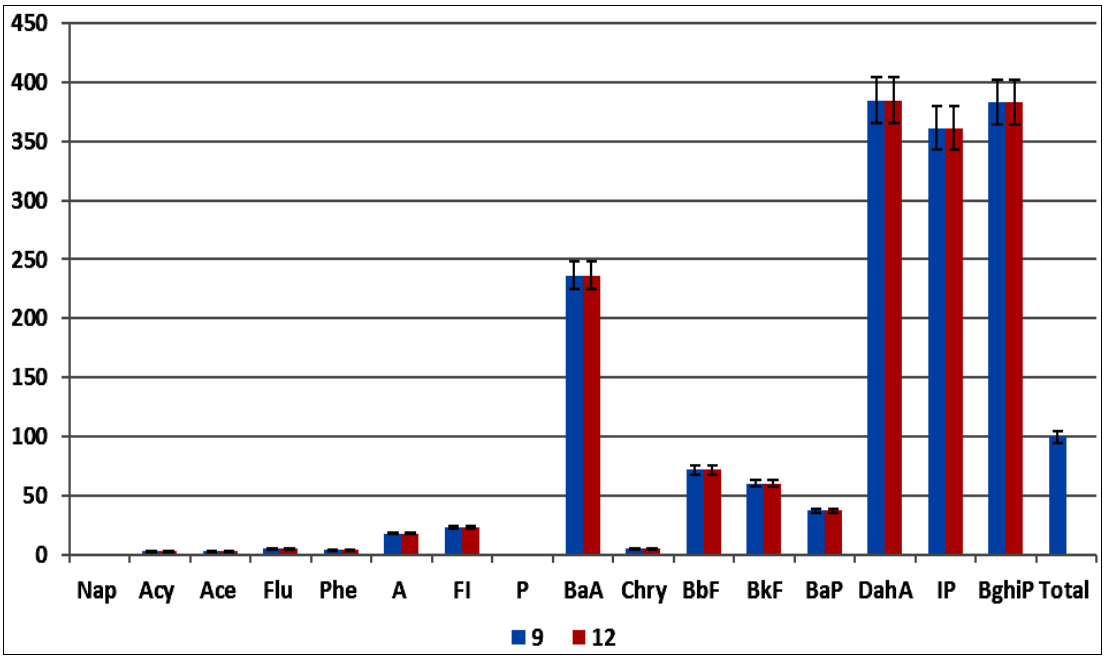


Fig 1: Logarithmic probit line for determination of 96-h LC_{50} crude oil and Diesel to *C. gariepinus*



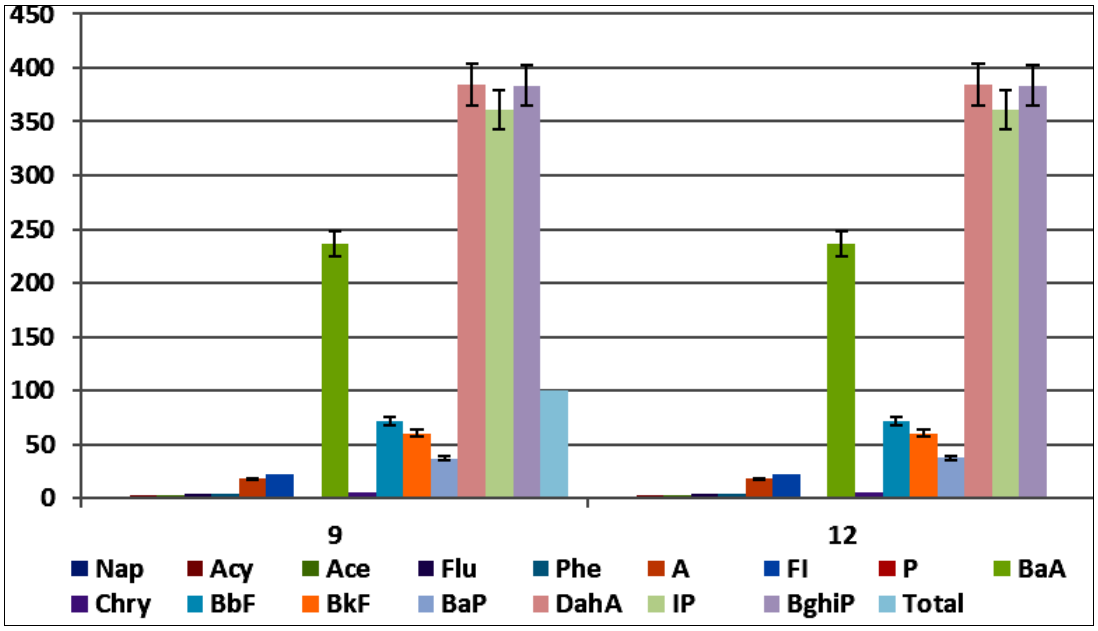
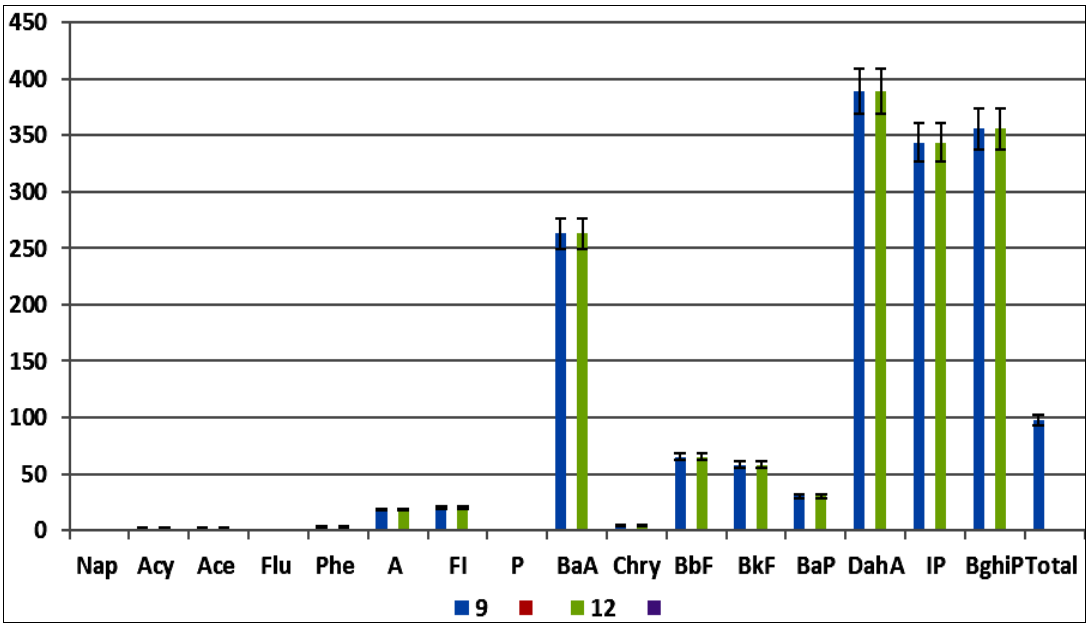


Fig 2: PAHs in fish exposed to concentrations of crude oil



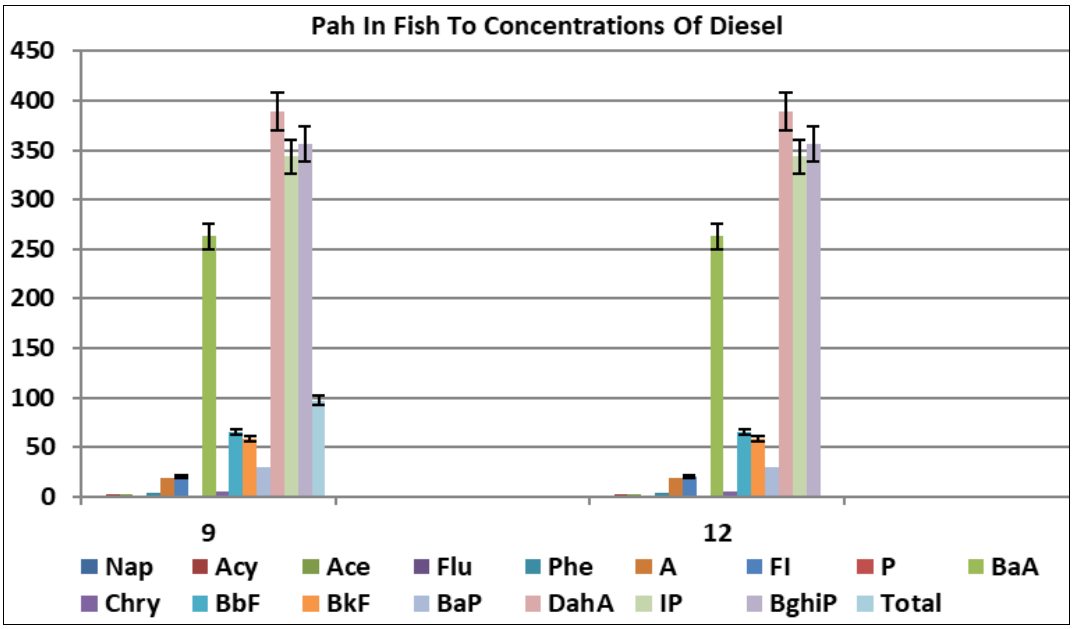


Fig 3: PAHs in crude oil and diesel

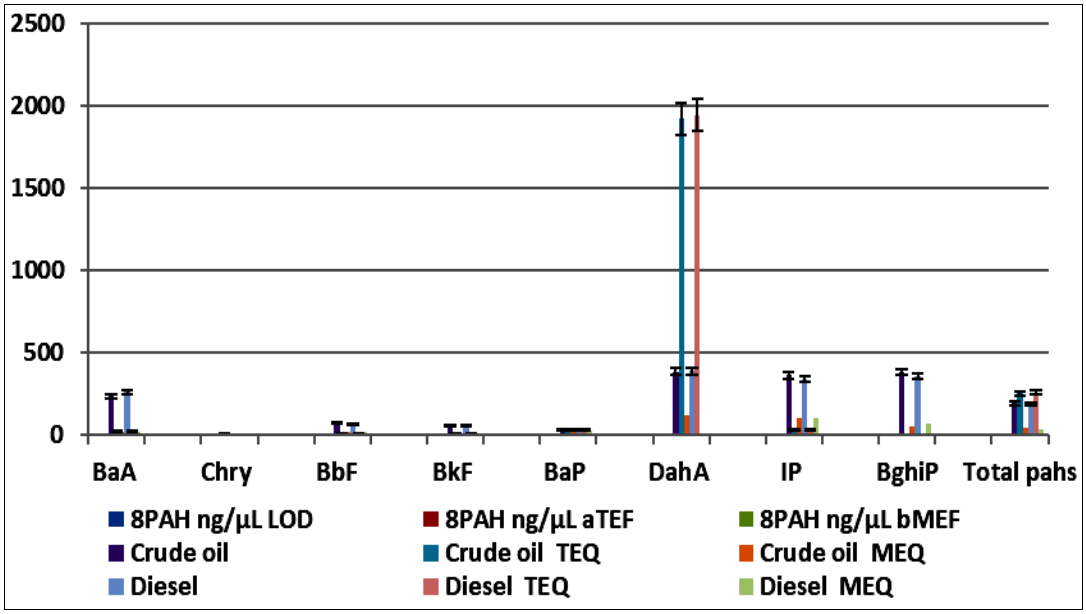
Different superscripts in a row indicate significant difference between means (ANOVA, $P< 0.05$)

KEY

cNap = Naphthalene, Acy= Acenaphthylene, Ace= Acenaphthene Flu= Fluorene, Phe=Phenathrene, A =Anthracene, FI =Fluoranthene P=Pyrene BaA=Benz {a} anthracene, Chry=Chrysene, BbF= Benzo [b] fluoranthene, BkF=Benzo [k] fluoranthene, BaP = Benzo [a] pyreneDahA = Dibenz [ah] anthracene IP = Indeno [123-cd] pyrene,, B[ghi]P = Benzo [ghi] pyrene, CO=Crude oil, P= Petrol, K= kerosene, D= Diesel, pp=petroleum products, c= concentration in ml/L, nd= not detected.

3.2 PAHs in exposed fish to petroleum

Total PAHs values in ng/µL (Figure 2) of petroleum products in exposed fish ranged from highest value of 99.68±4.81 crude oil > 97.30± 14.57 diesel oil ng/µL The lowest value of 0.061 was shown in Nap while highest value was 384.68 in DahA >383.47 B[ghi]P > 361.38 IP >236.41BaA >71.59 BbF>60.17 BkF >37.17 BaP> 22.82FI> 17.72A >5.33Chry >4.56 Flu> 4.35 Phe>2.79 Acy>2.40Ace >0.06Nap for crude oil.but P was not detected.. Diesel oil however indicated highest value of 389.05DahA> 355.70 B[ghi]P >343.48 IP>262.80 BaA>65.35 BbF>58.16 BkF>30.38 BaP>20.09 FI>18.48 A>4.79 Chry>3.79 Phe>2.51 Acy>2.22 Ace. P, Flu and Nap were not detected.



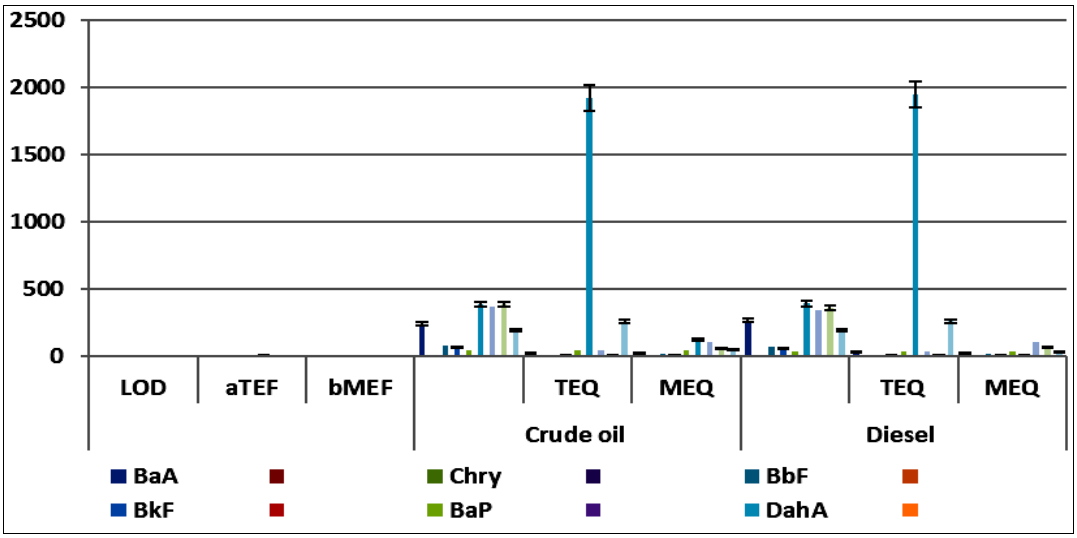


Fig 4: Bap-TEQ and Bap- MEQ of crude oil and diesel

TEF*: Toxic equivalency factors for cancer potency relative to BaP (Nisbet and LaGoy *et al*. 1992) [17].

MEF*: Mutagenic potency factor relative to BaP (Durant *et al*. 1996 and 1999) [5].

3.3 Carcinogenicity and mutagenicity equivalents

The least observed difference LOD of PAH among 8PAHs was 0.02ng/µL. Total TEQ and MEQ gave 254.67 and 44.84; 256.58 and 31.24 respectively for crude oil and diesel. BaP-TEQ in CO ranged from 0.05 in Chry to 1923 in DahA and 0.48 Chry-1.945 DahA respectively in Diesel. Similarly, BaP-MEQ ranged 0.09 Chry -119.25 DahA and 0.08 Chry-67.58 to BghiP in diesel.

4. Discussion

The result showed that diesel contained greater number of PAH than crude oil as well as being more carcinogenic and mutagenic, therefore was expected to be more toxic to the fish than petrol-treated ones. The contrary played out in our findings which gave the lowest value of toxicity level to be crude oil and therefore was more toxic compared to diesel. The indication that lighter petroleum products with lower total mean Σ16PAH showed greater toxicity on exposed fish than heavier ones having higher total mean Σ16PAH, probably due to greater level of more toxic BaA to BaP compared to less toxic but carcinogenic DahA- BaP in crude oil and DahA-BaP in diesel [2]. The impact of petroleum water soluble fraction previously under-reported has in recent times posed critical health concerns to aquatic biota, especially fish [8, 22]. The foregoing gave an indication that petroleum products with high molar mass and greater mean Σ8PAH were more carcinogenic and mutagenic compared to lighter petroleum with lower mean Σ8PAH. Crude and diesel oils have shown greater ability than crude oil to cause cancer and changes in the genetic makeup and may damage the genome materials or disrupt cellular metabolic processes of exposed fish to humans that consume them [9]. There is greater need for further investigation of the biochemistry exposed fish to these products since this approach may provide overestimation of cancer and mutagen potency of individual PAH, as most PAH indicated less comparative carcinogen than BaP.

Recent approaches have centered to identify and quantify PAHs in water, soil and air environment, their emission sources through various methods in order to evaluate their carcinogenic and mutagenic effects to human health [7, 19, 3]. The approaches distinguish anthropogenic multiple releases chiefly from petroleum and other sources [18, 9]. BaP is widely accepted as the indicator for measurement of carcinogenicity, thus BaP-equivalent toxicity for other carcinogenic PAHs has been recommended [28] and evaluated for cancer risk assessment [17, 5, 20, 27].

5. Conclusion

Crude oil was shown to be more toxic to the experimental fish but was less carcinogenic and mutagenic and had less number of pah. Further investigations on the biochemistry effects are required since over estimation of cancer and mutagen potencies may not be entirely ruled out.

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