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BaP-carcinogenicity and mutagenicity, growth and food reserves of clariid catfish to the water soluble fraction (WSF) of bonny light crude oil BLCO

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Abstract
The toxicity and PAH of *C. gariepinus* (0.98±0.01g, n=480) to 1, 3, 6, 9, 12 and 0.0 ml/L of BLCO oil was determined and were exposed to 3 treatment sublethal concentrations (2.40, 1.20, 0.60 (corresponding to 1/16, 1/8 and 1/4 of 96h LC_{50}) and 0.00 ml/L of WSF of BLCO in triplicate replications of 60 fish per treatment for 10 weeks. The 96h median lethal concentration of BLCO to the experimental fish fingerlings was 9.6 ml/L. The mean ∑ 16PAH gave 6% compared to 11% that of its mean 8PAH. Mean TEQ and MEQ in 8PAH both corresponded to 11%, however BaP-TEQ in BLCO ranged from 0% in Chry to 84% in DahA while BaP-MEQ ranged from 0% in Chry to 30% in DahA. The respective Benzo [a] Pyrene carcinogenic and mutagenic equivalents of *Clarias gariepinus* to BLCO was 2 and 9%. There was a significant difference ($P<0.05$) between mean weights, length and weight gain of exposed fish and control which depreciated along concentration gradient with the lowest growths at 2.4 ml/LWSF of crude oil. Mean weight gained by fish for the control group which stood at 0.24g reduced significantly by 31% to 0.07g in group exposed to 2.4ml/L WSF of BLCO. Mean FCR of the control group of fish with a value of 0.25 increased by 31% to 0.83 in exposed group to 0.24ml/L. liver glycogen and plasma glucose significantly reduced respectively by 16 and 8% to 0.64mg/g and 0.77mg/L at 2.4 ml/LWSF of crude oil- exposed fish which was indicative of hypoglycemia in the exposed fish.

Keywords: growth, *Clarias gariepinus*, water soluble fraction, crude oil, liver glycogen, plasma glucose

1. Introduction
The increasing incidence of oil pollution and a worldwide decline in protein ration necessitates the need for a database line of the PAH toxicity and effects of the water-soluble fractions of crude oil on the growth and food reserves of a widely distributed continental fish represented by *Clarias gariepinus*. The global preference for Bonny Light grade crude owing to its low sulphur content has increased production of the same in recent years in order to meet up with the high demand [29]. noted that the water-soluble fraction of crude oil which is non visible but solubilize and become bioavailable to fish and thus stay longer in water than other fractions.. In recent times, PAHs have received much attention due to their potential cause of cancer, mutagenic disorders and birth defects [20, 27]. The concentrations of petroleum products toxic to aquatic organisms depend on the type and hydrocarbon constituents, as well as the species involved [19]. 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 [25]. Fishes are good indicators of pollution due to the lipophilic nature and high chemical stability of PAHs which accumulate in the fatty tissues of fish after an uptake from both inland and coastal waters [16, 15, 11]. 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 [4].

The concentrations of petroleum products toxic to aquatic organisms depend on the type and hydrocarbon constituents, as well as the species involved [19, 18]. 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 (∑8PAH), having molecular weight greater than 228 gram. ∑8PAH 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) [24, 6, 7]. The hardy nature and possession of accessory air-breathing organs enable *C. gariepinus* to tolerate adverse aquatic conditions [28]. 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 toxicity and levels of PAH of Nigerian-petroleum crude oil effects on the growth and food reserves of *C. gariepinus* fingerlings.

2. Materials and Methods

2.1. Experimental fish and petroleum

A total of one hundred and twenty (180) 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) was obtained from Nigerian National Petroleum Cooperation Enugu. The water soluble fraction WSF was prepared following the method of [33], 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 crude oil 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 (60) 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 in triplicateto determine 96h LC_{50} using the probit analysis proposed by [12, 34, 35] and polycyclic aromatic hydrocarbons (PAH) in exposed fish [19]. The exposure 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 [3, 36] 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 were sampled to determine ∑16PAH, ∑8PAH, TEQ and MEQ. A portion of the sample using the GC-MC was taken for extraction and analysis of PAH [32, 22].

2.3 PAH extraction

The method described by [32, 17, 20] 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 [24, 31, 7]. The BaP carcinogenic equivalent (BaP_{TEF}) and BaP mutagenic equivalent (BaP_{MEQ}) for the individual PAHs was calculated: $BAP_{TEQ} = \sum C_1 \text{ X } BAP_{TEF}$; $BAP_{MEQ} = \sum C_1 \text{ X } BAP_{MEF}$, where Ci = concentration of PAHs.

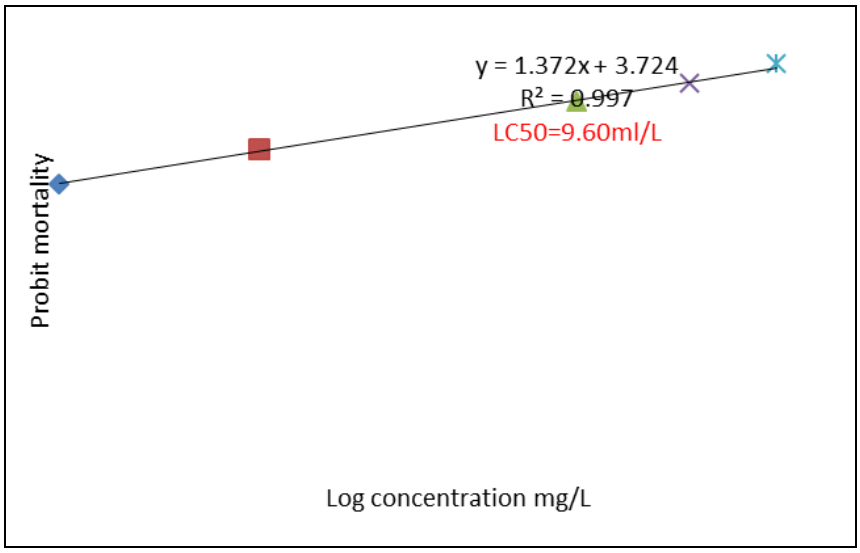
2.4 Subacute Toxicity Test

Three sublethal concentrations 2.40, 1.20, 0.60 (corresponding to 1/16, 1/8 and 1/4 of 96h LC_{50}) and 0.00 ml/L of WSF of BLCO in triplicate replications of 60 fish per treatment for 10 weeks was determined in 18 plastic vats, each containing 20 fingerlings of *C. gariepinus*. Exposure lasted for 10 weeks during which 2 juveniles were sacrificed per aquarium at fortnightly intervals to estimate carbohydrate food reserves. Feeding was conducted at 3% body weight twice daily but the fresh introduction of toxicant and syphoning of faecal matter and uneaten food was carried out weekly. Water quality parameters as determined during the acute toxicity test were conducted fortnightly following the method of [3]. The determination of plasma glucose and liver glycogen content were carried out by comparing the absorbance of anthrone portions with those of standard glucose using a colorimeter (Model 605/REV D/01-96) at 620 nm following the method suggested by [38]. Diet with 38% crude protein was formulated using the Pearson Square Method [23]. It was used to feed juveniles of *Clarias gariepinus* during the acclimation period and subacute exposure periods at 3% body weight, twice daily. Ingredient consisted of soybean meal, yellow maize, fish meal, rice bran, salt, palm oil, vitamin mix and mineral mix. These ingredients were carefully weighed out using Ohaus digital weighing balance (model Adventurer ProAV410C). This was followed by grinding of the micro-ingredients, then mixed in a mixer and pelleted with a pelleting machine. Pelleted diet was allowed to dry before packaging and usage during acclimatization and subacute exposure periods. Proximate Analyses of formulated diet was carried out according to the methods of [2] for moisture content, crude protein, ether extracts (fats), Ash content, Nitrogen free extract while water quality was estimated using the methods of [3].

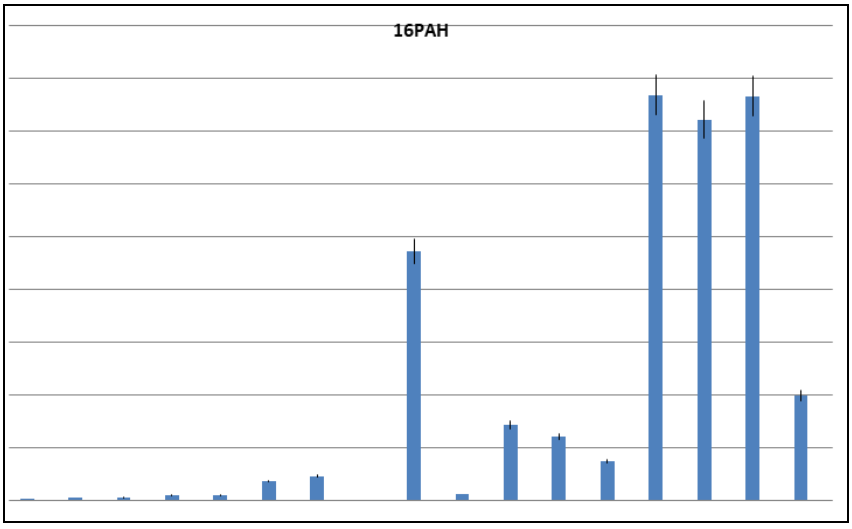
2.5 Statistical Analysis

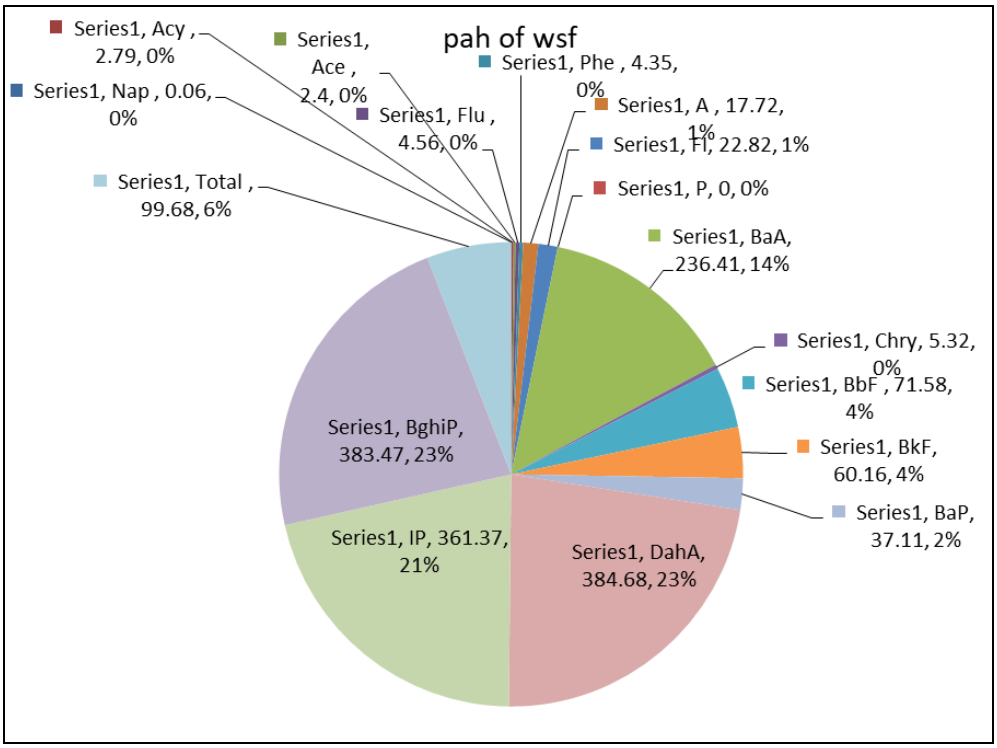
Data obtained were expressed as standard mean ± standard error of mean and analyzed using the statistical package SPSS 20.0 computer program (SPSS Inc. Chicago Illinois, USA.

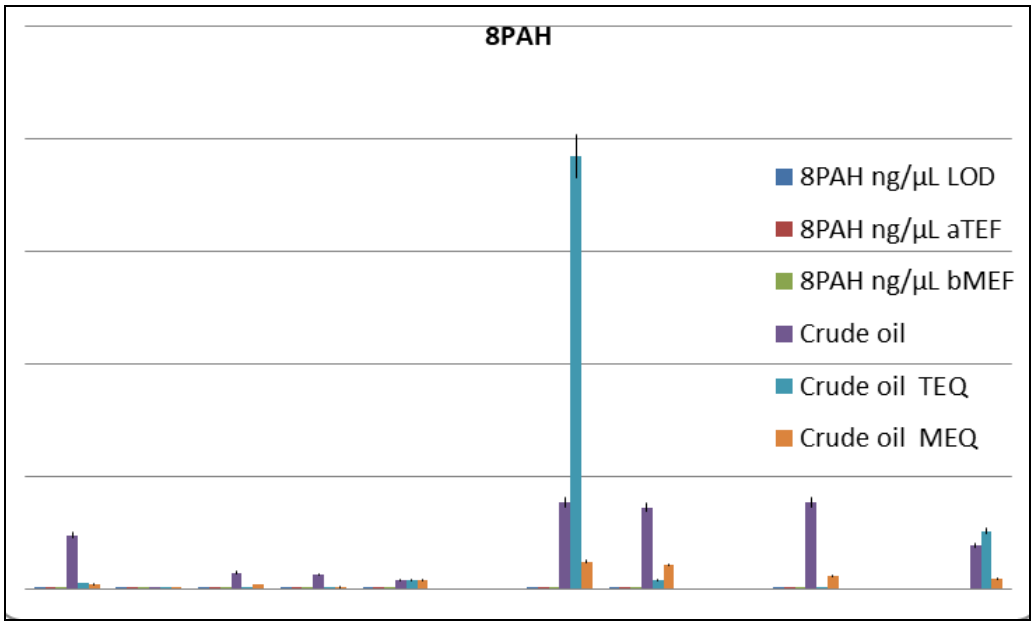
3. Results

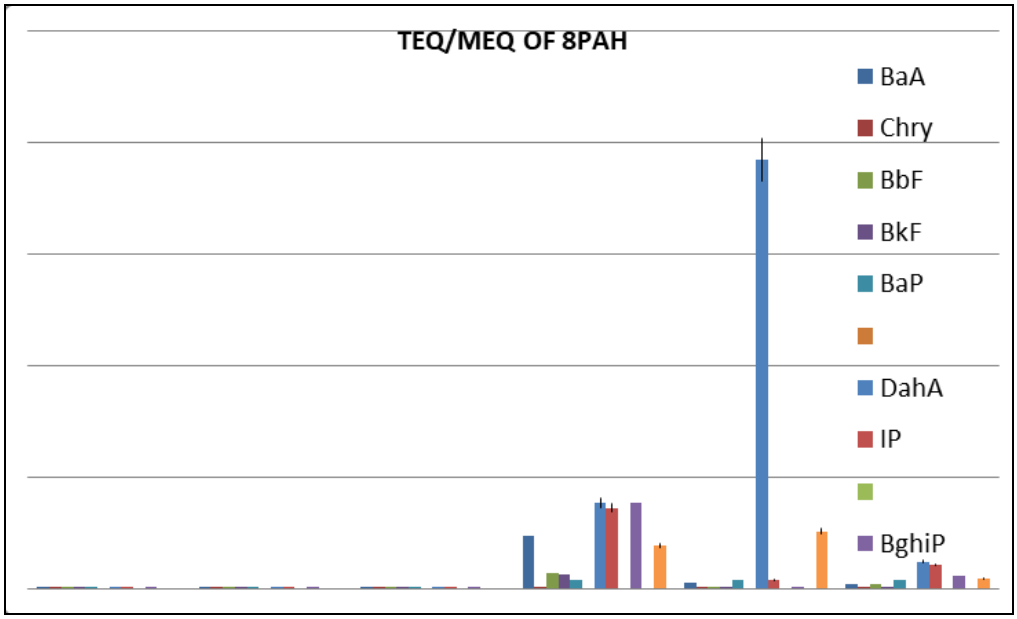


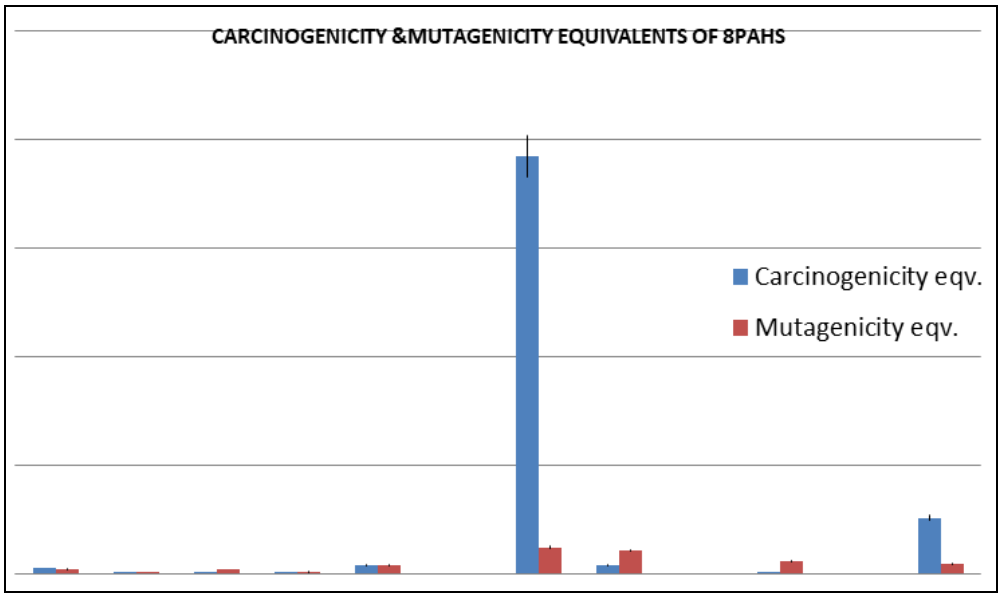
Logarithimic probit line for determination of 96-h LC_{50} of BLCO to *C. gariepinus*

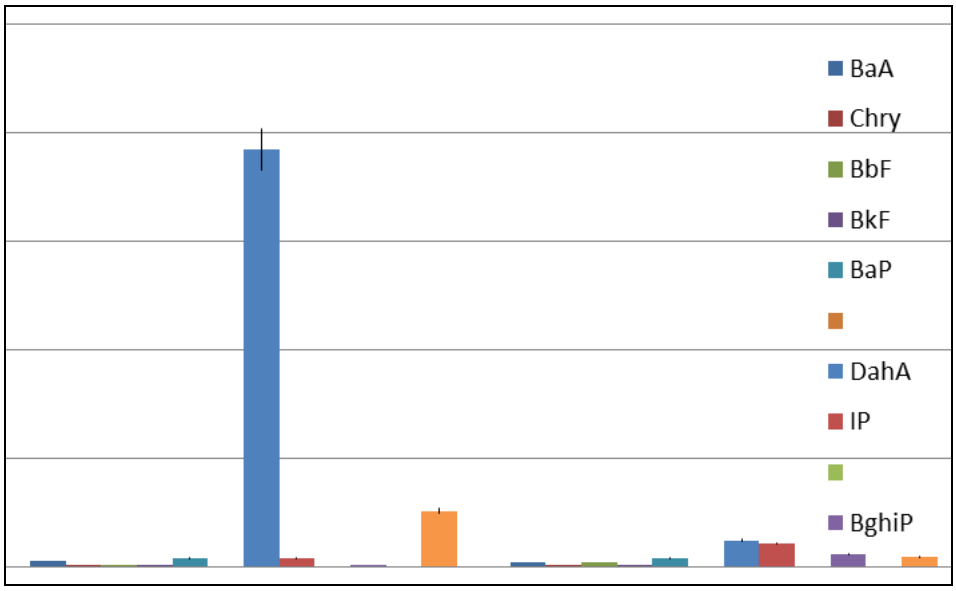


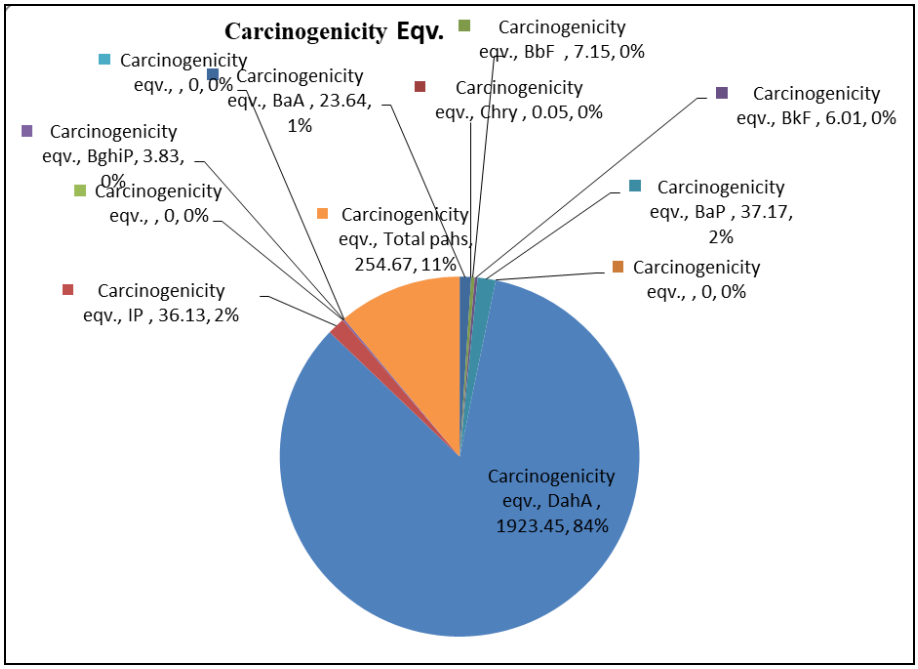


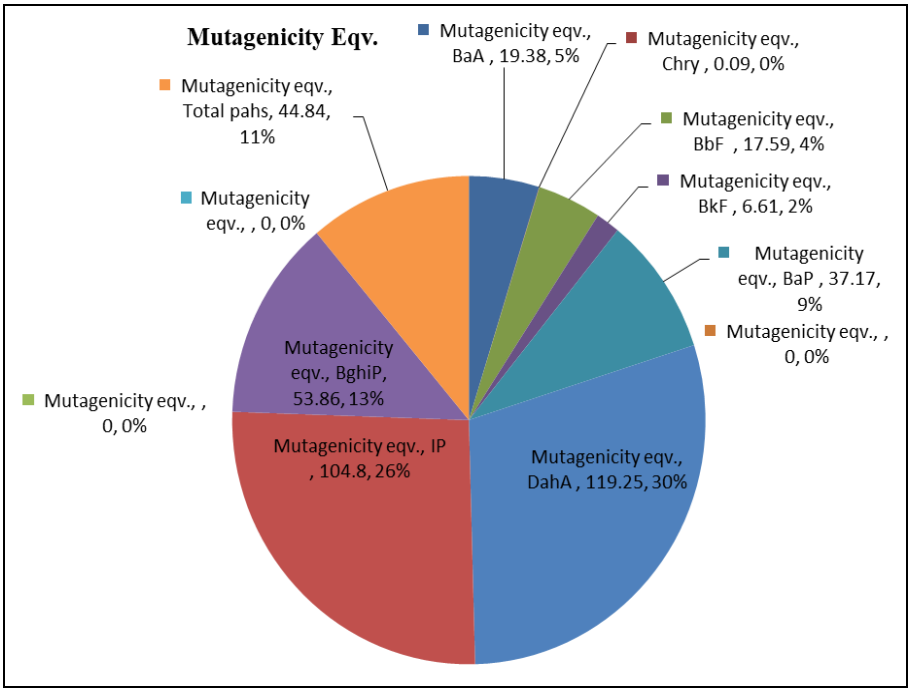

8PAH
8PAH ng/µL LOD
8PAH ng/µL aTEF
8PAH ng/µL bMEF
Crude oil
Crude oil TEQ
Crude oil MEQ


TEQ/MEQ OF 8PAH
BaA
Chry
BbF
BkF
BaP
DahA
IP
BghiP


CARCINOGENICITY &MUTAGENICITY EQUIVALENTS OF 8PAHS
Carcinogenicity eqv.
Mutagenicity eqv.


BaA
Chry
BbF
BkF
BaP
DahA
IP
BghiP


Carcinogenicity Eqv.
Carcinogenicity eqv., , 0, 0%
Carcinogenicity eqv., BaA , 23.64, 1%
Carcinogenicity eqv., BbF , 7.15, 0%
Carcinogenicity eqv., Chry , 0.05, 0%
Carcinogenicity eqv., BkF , 6.01, 0%
Carcinogenicity eqv., BghiP, 3.83, 0%
Carcinogenicity eqv., , 0, 0%
Carcinogenicity eqv., Total pahs, 254.67, 11%
Carcinogenicity eqv., BaP , 37.17, 2%
Carcinogenicity eqv., , 0, 0%
Carcinogenicity eqv., IP , 36.13, 2%
Carcinogenicity eqv., DahA , 1923.45, 84%


Mutagenicity Eqv.
Mutagenicity eqv., BaA , 19.38, 5%
Mutagenicity eqv., Chry , 0.09, 0%
Mutagenicity eqv., Total pahs, 44.84, 11%
Mutagenicity eqv., BbF , 17.59, 4%
Mutagenicity eqv., BkF , 6.61, 2%
Mutagenicity eqv., , 0, 0%
Mutagenicity eqv., BaP , 37.17, 9%
Mutagenicity eqv., , 0, 0%
Mutagenicity eqv., BghiP, 53.86, 13%
Mutagenicity eqv., , 0, 0%
Mutagenicity eqv., IP , 104.8, 26%
Mutagenicity eqv., DahA , 119.25, 30%

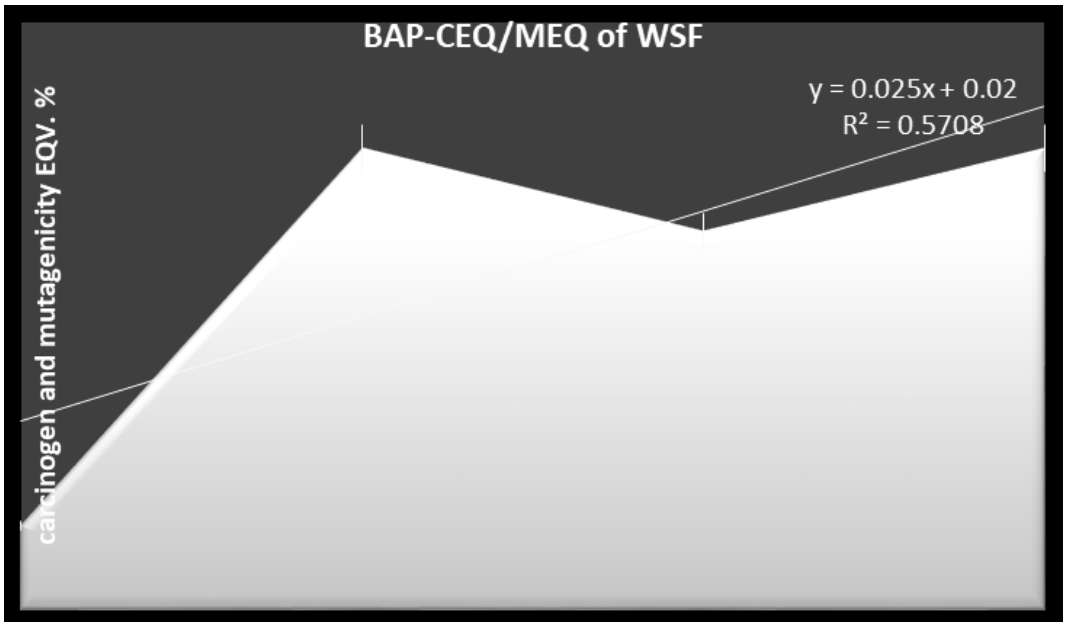


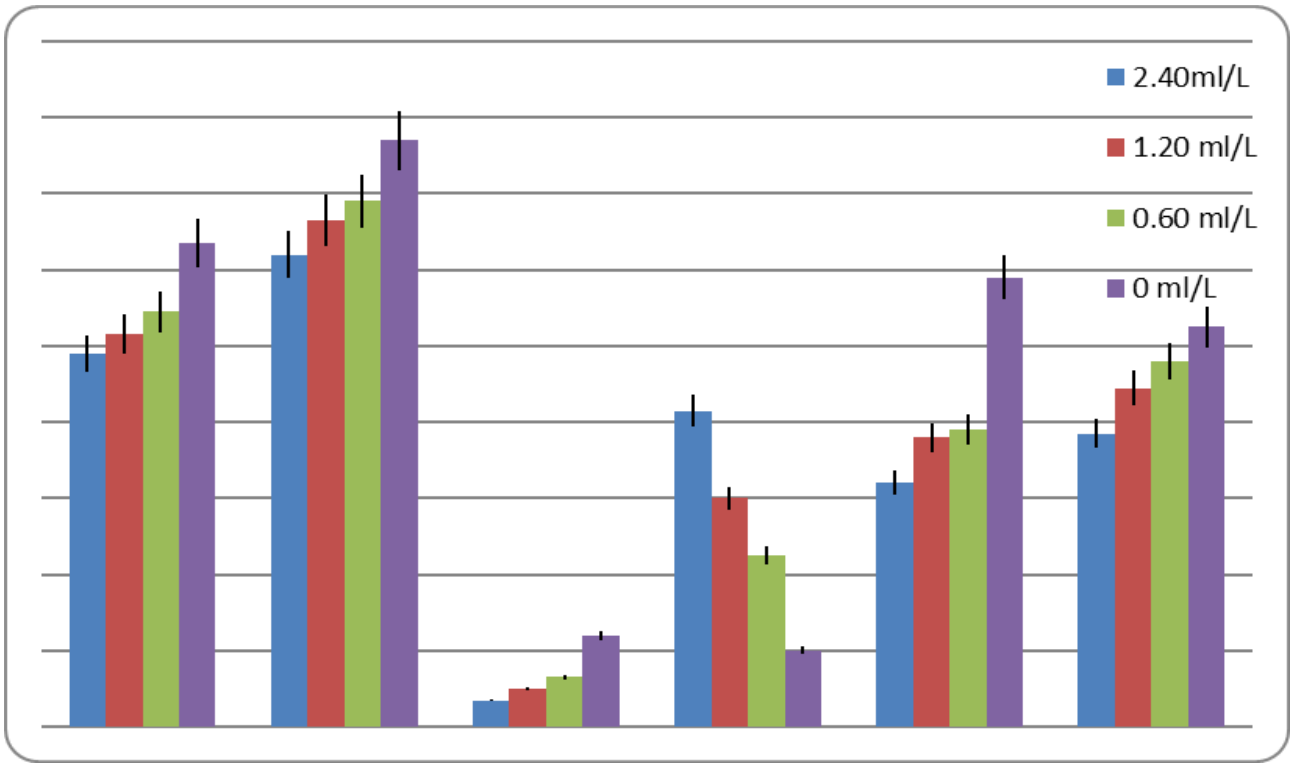
Fig 1: 16 & 8 PAH; CEQ & MEQ of *C. gariepinus* to BLCO

The results of the mean growth of *Clarias gariepinus* exposed to sublethal Concentrations of WSF of Crude oil is presented in tables and figures below

Table 1: Mean Growth and food reserves of *Clarias gariepinus* to WSF of crude oil

Parameters	2.40ml/L	1.20 ml/L	0.60 ml/L	0.00 ml/L
Mean weight (g)	0.985 ± 0.34^{b}	1.03 ± 0.40^{b}	1.09 ± 0.65^{b}	1.27 ± 0.17^{a}
Mean length (cm)	1.247 ± 0.38^{b}	1.33 ± 0.64^{b}	1.38 ± 0.68^{b}	1.54 ± 0.13^{a}
Mean weight gain (g)	0.07 ± 0.003^{c}	0.1 ± 0.004^{b}	0.13 ± 0.003^{b}	0.24 ± 0.004^{a}
FCR	0.83^{b}	0.60^{c}	0.45^{c}	0.25^{d}
Liver glycogen	0.64 ± 0.04^{c}	0.76 ± 0.05^{b}	0.78 ± 0.05^{b}	1.18 ± 0.02^{a}
Plasma glucose	0.77 ± 0.01^{d}	0.89 ± 0.01^{c}	0.96 ± 0.02^{b}	1.05 ± 0.05^{a}

Means on a row with the same superscript are not significantly different but means with different superscript are significantly different. Mean separation by Duncan's Multiple Range Test at 5% level of significance (P>0.05).



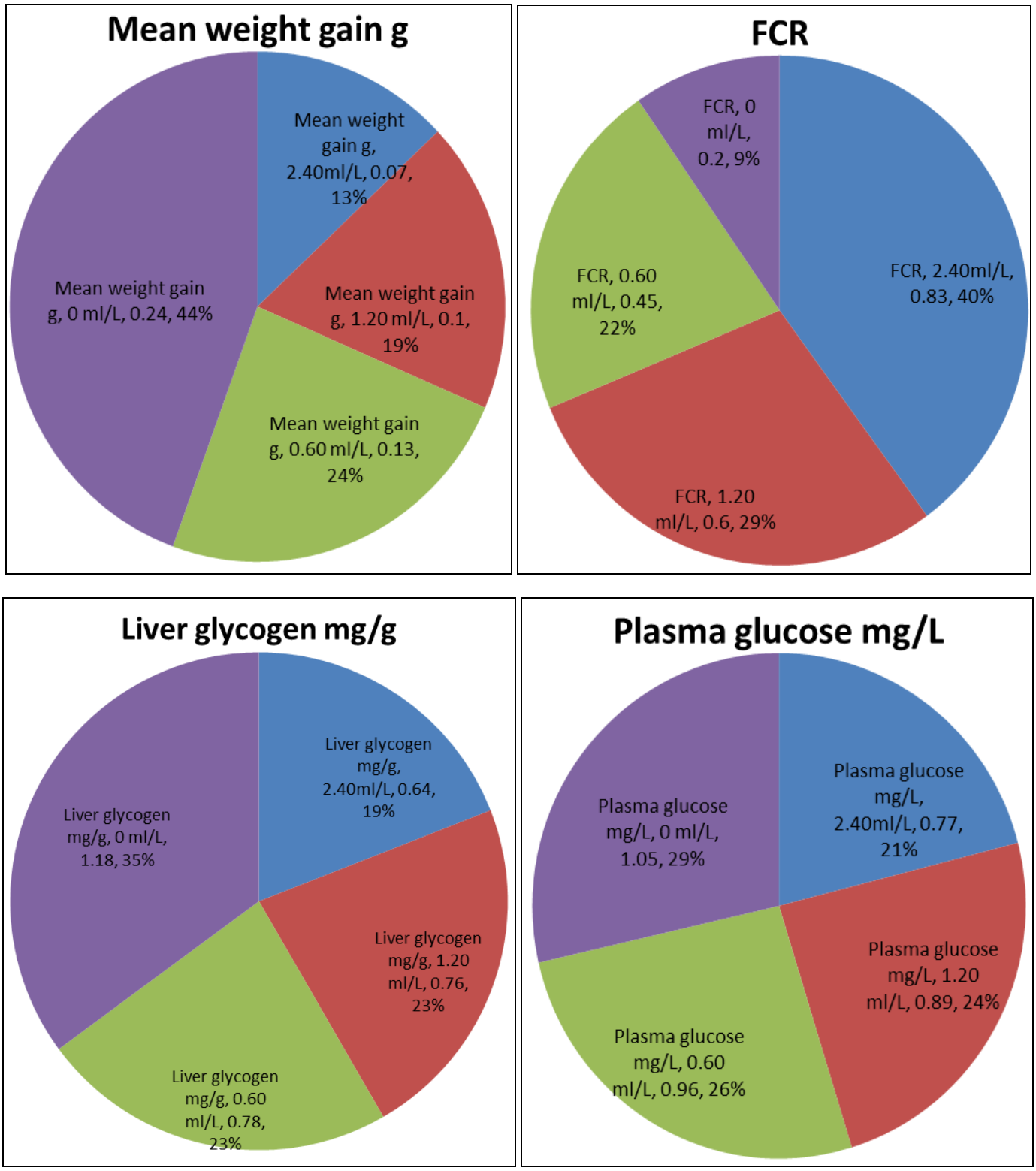


Fig 2: Growth and food reserves of *C. gariepinus* to sublethal concentrations of BLCO

3.1 Growth of *Clarias gariepinus*

There was a significant difference ($P<0.05$) between mean weight, length and weight gain of exposed fish and control which depreciated along concentration gradient with the lowest growths at 2.4 ml/LWSF of crude oil. Mean weight gained by fish for the control group which stood at 0.24g reduced significantly by 31% to 0.07g in group exposed to 2.4ml/L WSF of BLCO. Mean FCR of the control group of fish with a value of 0.25 increased by 31% to 0.83 in exposed group to 0.24ml/L. liver glycogen and plasma glucose significantly reduced respectively by 16 and 8% to 0.64mg/g and 0.77mg/L at 2.4 ml/LWSF of crude oil- exposed fish which was indicative of hypoglycemic alterations in the exposed fish.

Table 2: Gross & Proximate analysis of Diet

Ingredients % of crude protein inclusion	
Yellow maize (10%)	9.28
Soybean meal (40%)	54.85
Fish meal (64%)	16.75
Rice bran (8%)	15.37
Micronutrient inclusion	
Salt	0.25
Palm oil	0.5
Vitamin mix	0.6
Mineral mix	2.4
Total	100
Proximate Analysis of Formulated Diet	
Crude protein	37.8
Ether extracts	0.57
Ash	5.69
Moisture	8.59
Nitrogen-free extract	45.55
Crude fibre	1.8
Total	100

4. Discussion

The WSF of BLCO has shown the ability to cause more changes in the genetic makeup and may damage the genome materials than its ability to cause cancer in exposed fish [14]. The impact of petroleum water soluble fraction previously under-reported has in recent times posed critical health concerns to aquatic biota, especially fish [13, 30]. The foregoing gave an indication that petroleum products with high molar mass and greater mean $\sum$ 8PAH were more carcinogenic and mutagenic compared to lighter petroleum with lower mean $\sum$ 8PAH. 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 [12, 26, 5]. The approaches distinguish anthropogenic multiple releases chiefly from petroleum and other sources [25, 14]. BaP is widely accepted as the indicator for measurement of carcinogenicity, thus BaP-equivalent toxicity for other carcinogenic PAHs has been recommended [39, 4] and evaluated for cancer risk assessment [24, 7, 27, 37]. The result showed that BLCO contained greater percentage mean of 8PAH than 16PAH and was less carcinogenic than mutagenic to exposed group of *C. gariepinus*. There is greater need for further investigation of the biochemistry and physiology on the mutagenicity to both fish, animals and humans alike that consume them given high mutagenicity responses of the experimental fish to BLCO in the present research. The lowered mean weight gain of exposed fish to WSF of BLCO compared to the control group agreed with previous reports of [21, 1] when they exposed same species to petroleum and ultraviolet radiation. Toxicant effect hindered food intake and resulted to increased rate of food conversion which possibly led to poor growth among exposed groups of fish. Some authors noted that petroleum impairment of carbohydrate metabolism in test fish was due to allocation of greater energy to the breakdown of aromatic hydrocarbon and excretion of the same which lead to reduction in growth among the exposed fish [10,9]. Thus water-soluble fractions of crude oil stimulated hydrocarbon metabolism at the expense of tissue growth which might have lead to poor growth among exposed group than control fish [10, 8]. The present research is suggestive that the WSF of BLCO elicited hypoglycemic condition in test fish.

5. Conclusion

The respective Benzo [a] Pyrene carcinogenic and mutagenic equivalents of *Clarias gariepinus* to BLCO was 2 and 9%. Mean weight gained by fish for the control group which stood at 0.24g reduced by 31% while Mean FCR of the control group of fish increased by the same rate to the exposed group at the highest concentration. liver glycogen and plasma glucose significantly reduced respectively by 16 and 8% from control to the highest exposed group and thus elicited hypoglycemic alterations in the exposed fish.

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