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Bioaccumulation of polycyclic aromatic hydrocarbons from light and heavy crude oils in fingerlings of the African catfish (*Clarias gariepinus*)

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ABSTRACT

Crude oil spillage, although not so common these days, introduces various chemicals that are toxic to the environment and pose different health risks to the exposed organisms, including humans. The bioaccumulation of light and heavy crude oils in fingerlings of the African catfish (*C. gariepinus)* was investigated by acute toxicity test and GC–MS. The fishes were exposed to different volumes of the oils for different duration, ranging from 24 to 96 h. The dissolved oxygen (DO), pH and temperature were monitored to establish the quality of the water in the tanks. The results showed that the quality of the water reduces as the percentage of crude oils in the 20 L of the treatment solution increases and the exposure time increases, posing difficult living conditions to the fishes. In the same manner, fish mortality increases with the concentration of oils and exposed time. The detected concentrations of PAHs in dead fishes were low which may be due to the low volume of crude oils, the volatile nature of the oils and the excretion of metabolic waste by the fishes. The high mortality rate was attributed to high toxicant volume (ET_3–ET_5, especially in light crude oil) and high exposure time (72–96 h). Although, the PAHs concentrations were low (0.001 - 0.680 mg/kg), a further study to establish the concentration of PAHs contributed by the crude oils is recommended.

HIGHLIGHTS

- Bioaccumulation of PAHs in fish contaminated with crude oil was studied.
- Both light and heavy crude oil were investigated.
- The nine (9) PAHs studied were detected.
- The concentration of PAHs increases with increasing crude oil in 20 L water.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) belong to the group of organic compounds containing two or more aromatic rings joined together (Nam *et al*., 2021; Javed *et al*., 2019). PAHs are sourced from both natural and artificial origins especial from incomplete combustion of petroleum products (about 90% of PAHs) like vehicular fuel, cooking gas, and burning of crude oil spillage (Wangboje & Opobo, 2019). PAHs may get to humans through inhalation, dermal contact and uptake in diet (Nam *et al*., 2021). There are many causes of oil spillage, which include accidental spillage, leaching from transporting ships and pipelines, sabotage, and pipelines vandalism among others, and their effects are enormous on the environment and exposed humans (Omoregie & Ufodike, 2000; Adeyemi, 2004). Oil spillage is generating concern due to the negative impact it has on the environment, humans and the economy, and serious attention is being paid to it, especially to mitigating effects on biodiversity.

Crude oil is toxic due to the presence of water-soluble and volatile organic substances in it (Idodo-Umeh & Oronsaye, 2005), and it is classified based on its relative density as light or heavy crude oil according to American Petroleum Institute (API) gravity criteria. Light crude oil refers to petroleum with low density at room temperature, low viscosity, low specific gravity and high API gravity (more than 20°) due to the presence of a high proportion of light hydrocarbon fractions while heavy crude oil means any liquid petroleum with an API gravity less than 20° (Dusseault, 2001).

In Nigeria, the African catfish (*Clarias gariepinus*) is a commercial species of importance due to its wider acceptability by consumers, oily flesh and nice taste (Akande & Ajayi, 2002). Due to a large number of crude oil spills recorded, so far, in Nigeria, especially Niger Delta areas, most pollution studies on edible aquatic creatures have reported the effects these oils have on fingerlings (Nwadukwe & Ayinla, 2004; Nwadukwe *et al*. 2004; Nwadukwe *et al*., 2006) hence, the choice of the fingerlings of the African catfish (*Clarias gariepinus*). Effects of heavy metals, crude oils, as well as its fractions on Nile tilapia (*Oreochromis niloticus)*, matured

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African catfish (*Clarias gariepinus*) (Dede & Kaglo, 2001; Adeboyejo, *et al*., 2013), *Heterobranchus bidorsalis* (Nwabueze & Agbogidi, 2010), common carp (*Cyprinus carpio*) have also been reported (Rajeshkumar *et al*., 2017). However, there is a dearth of information on the bioaccumulation of PAHs caused by light and heavy crude oils. Thus, this study investigated nine bioaccumulated PAHs from light and heavy crude oils in fingerlings of the African catfish (*Clarias gariepinus*) acquired from South-southern Nigeria—a region most polluted with petroleum products in the country—using GC-MSD.

2.0 Materials and Methods

2.1 Materials and Reagents

Crude oil used to modify the stocking media was obtained from the Nigerian Petroleum Development Company (NPDC), Oredo Field, Edo State, Nigeria. Agilent Gas Chromatograph, HP 5890 Series 11® with mass selective detector (MSD) were used to analyze the organic congeners. Glass aquaria tanks (20 L) with dimension (50x25x26 cm^3) were used for the bioassay, Panasonic® MX-J210N electric blender was used to blend dried sample to powder and vacuum rotatory evaporator was used to evaporate extracted sample. Other apparatus used include Monofilament netting, Mettler weighing balance (Ohaus Corporation, USA model: scout pro SPLI 401 series), 9-series multi-water quality meter (Hanna Instrument), sintered funnel, and extraction bottles with Teflon covers. Analytical standard grade reagents and chemicals including anhydrous sodium sulphate (Na_2SO_4, 99%), silica gel, acetone (98%), dichloromethane (DCM, 98%) and H_2SO_4 (98%) were purchased from Labwaco Chemicals, Benin City, Nigeria. The Na_2SO_4 was dried in a muffle furnace for 3 h at 450 °C and kept in a desiccator (in an amber glass bottle) before use. Standard of PAHs containing naphthalene (NAP), 2-methylnapthalene (2-MN), acenaphthene (ACN), acenaphthylene (ACL), phenanthrene (PHT), anthracene (ANT), fluoranthene (FLT), benzo(a)pyrene (B(a)P) and dibenzo [a,h] anthracene (D(ah)A) was purchased from Labwaco Chemicals, Benin City, Nigeria to prepare GC-MS working solution.

2.2 Collection of Fish Samples

A total of four hundred and thirty-two (432) healthy *C. gariepinus* fingerlings of apparently nine (9) weeks old, mean length 15.40±0.57 cm and mean weight 22.08±2.51 g were purchased from Jossy Farms, Benin City, Edo State, Nigeria and transported to the laboratory in well-aerated oxygenated bags. They were held in thirty-six (36) open-top glass aquaria tanks containing 20 L of clean borehole water for acclimation to laboratory conditions and fed to satiation with 2 mm Coppens fish feed for a week, and the water was renewed daily to prevent buildups of waste from the unconsumed feed.

2.3 Range–Finding and Definitive (acute toxicity) Tests

A range-finding test was carried out in six (6) treatment setups containing 0.0% and 1.0, 1.5, 2.0, 2.5 and 3.0 % of light crude oil in a 20 L rectangular glass tank, first, and repeated with the same percentage concentrations of heavy crude oil (American Public Health Association [APHA], 1971; Solbe, 1995; Omitoyin *et al*., 2006; Akin-Obasola, 2019). Monofilament nettings were used to cover the tanks to prevent the fish from jumping out, and the setups were labelled ET_0, ET_1, ET_2, ET_3, ET_4 and ET_5 for blank and the concentrations of the oils, respectively. Exactly 12 pieces of *Clarias gariepinus* fingerlings were transferred to each tank, and the experiments were monitored at 3 h intervals and lasted for 72 h (Ward *et al*., 1982; Akin-Obasola, 2019) and mortality was recorded as a function of the failure to respond to external stimuli. The procedure was repeated for 96 h to determine the acute toxicity of the oils, and mortality was monitored at every 3 h. Temperature, pH and dissolved oxygen level of the water media were monitored using the multi-water quality meter (Abowei *et al.,* 2005; Akin-Obasola, 2019). Experimental procedures were repeated in triplicate.

2.4 Exposure Procedure

The range finding experiment was repeated in triplicate 0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 % of light crude oil to determine the amount of PAHs and SAHs bioaccumulated in the fishes, and the procedure was repeated for heavy crude oil. Here, mortality was monitored at a regular interval of 24 h, and dead fishes were collected, stored in an ice-shelve cooler and subjected to further laboratory analysis to determine the concentrations (µg/kg) of PAHs.

2.5 Dissection procedure

The dead fishes were thawed, and the flesh was removed, oven-dried at 85 °C for 2 h in a muffle furnace and milled to powder using the blender. Powdered flesh was transferred to amber bottles and kept at -20 °C.

2.6 Extraction and clean-up

The bioaccumulated PAHs and SAHs were extracted from the powdered fish flesh using the cold extraction method as described by Dean and Xiong (2000). Briefly, xx mL of DCM and acetone (1:1 v/v) was transferred into an extraction bottle and about 10.0 g of the fish was added, covered with Teflon, shook for about 5 min and filtered using the sintered funnel. The extraction procedure was repeated in triplicate and the filtrate was cleaned using activated silica gel and concentrated to about 2 mL using the vacuum rotary evaporator. The solvent was exchanged to DCM and transferred to a 5 mL amber bottle and kept at –20 C before analysis.

2.7 Instrumental analysis of PAHs and SAHs

An Agilent 6890 gas chromatograph (GC) interfaced with an Agilent 5973N Mass Spectrometer (MS) was used in this study. The extracts were injected in the splitless mode with the injector port temperature at 300 C using an automatic liquid sampler injector. Separation was carried out using a META X5 coated fused capillary column (30 m × 0.25 mm ×0.25µm) with

helium as carrier gas (1 mL min^{-1}). The column temperature was programmed from 70 °C (initial equilibrium time of 2min) to 300 °C at a rate of 20 °C/min, maintain for 15 min, yielding a total run-time of 39.50 min. The MS source and MS Quad temperatures were maintained at 230 °C and 150 °C, respectively, and the transfer line and ion source temperatures were set at 280 °C. The mass spectra were taken with a scan range of 50 –550 amu while the ionizing energy was 70 eV and the electron multiplier voltage was obtained from auto-tune. The limit of detection (LOD) and limit of quantification (LOQ) of the method are in the range of 0.05–0.2 µg kg^{-1} and 0.08–0.840 µg kg^{-1}, respectively.

2.8 Statistical analysis

A GENSTAT® computer software (version 12.1 for windows) was used for statistical analysis. Data were subjected to Analysis of Variance (ANOVA) to determine significant differences between mean concentrations of PAHs from light and heavy crude at 5% level of significance. Significant means were separated using Duncan Least Significant Difference (LSD) (Duncan, 1955).

Table 1. Quality Parameters of Water samples in the *C. gariepinus* fingerlings ponds**.**

Concentration (µg/L)	DO (mg/L)		pH		Temperature (°C)	
	LCO	HCO	LCO	HCO	LCO	HCO
ET_0	6.20^a	6.24^a	6.67^a	6.76^b	27.57^a	27.72^a
ET_1	4.65^b	4.76^b	6.31^c	6.62^d	26.61^b	26.96^b
ET_2	4.45^c	4.45^c	6.27^e	6.62^f	26.62^c	27.12^c
ET_3	4.00^d	4.13^e	6.19^g	6.59^h	26.66^d	27.31^d
ET_4	3.62^f	3.49^g	5.95^i	6.56^j	26.83^e	27.32^e
ET_5	2.95^h	2.55^i	5.94^k	6.50^l	26.86^f	27.33^f
LSD	0.123		0.029		0.901	
SED	0.062		0.015		0.451	

Means values with the same superscripts are not significantly different (P>0.05), horizontally; LCO = Light Crude Oil, HCO = Heavy Crude Oil, LSD = Least Significant Difference, SED= Standard Error of the Difference between two means.

Table 2. *C. gariepinus* fingerlings mortality caused by LCO and HCO from both range-finding and definitive (acute toxicity) tests

Time elapsed (h)	Toxicant	ET_0	ET_1	ET_2	ET_3	ET_4	ET_5
24*	LCO	0	2±0.6	5±0.6	8±0.6	10±0.6	12±0.0
48*		0	4±1.0	6±0.0	10±0.6	11±0.6	12±0.0
72*		0	5±1.0	7±0.6	12±0.0	12±0.0	12±0.0
96**		0	7±0.6	7±0.6	12±0.0	12±0.0	12±0.0
24*	HCO	0	2±0.0	4±0.6	6±0.0	7±0.6	10±0.6
48*		0	3±0.6	5±0.6	7±0.6	8±0.6	10±0.6
72*		0	4±0.6	5±0.6	8±0.6	9±0.0	11±0.6
96**		0	5±0.6	7±0.6	9±0.0	11±0.6	12±0.0

* = results from both range-finding and definitive (acute toxicity) tests; ** = results from definitive (acute toxicity) test only

Table 3. Mean Concentration of PAHs Cogeners from Light and Heavy Crude Oils after Exposure in *C. gariepinus* fingerlings

PAHs	Concentration(µg/kg)											
	ET_0		ET_1		ET_2		ET_3		ET_4		ET_5	
	LCO	HCO	LCO	HCO	LCO	HCO	LCO	HCO	LCO	HCO	LCO	HCO
NAP	BDL	BDL	0.006±0.03	0.047±0.05	0.021±0.02	0.001±0.02	0.033±0.01	0.0027±0.001	0.045±0.02	0.035±0.01	0.045±0.03	0.048±0.03
2-MN	BDL	BDL	0.002±0.01	0.344±0.03	0.020±0.01	0.022±0.07	0.024±0.12	0.027±0.001	0.047±0.02	0.036±0.01	0.295±0.02	0.059±0.03
ACN	BDL	BDL	0.001±0.01	0.005±0.03	0.079±0.05	0.069±0.03	0.680±0.04	0.078±0.003	0.099±0.04	0.098±0.04	0.099±0.02	0.103±0.01
ACL	BDL	BDL	0.001±0.00	0.018±0.02	0.007±0.01	0.053±0.04	0.009±0.03	0.037±0.002	0.009±0.03	0.048±0.02	0.010±0.01	0.099±0.04
PHT	BDL	BDL	0.025±0.02	0.056±0.03	0.042±0.02	0.329±0.05	0.355±0.33	0.338±0.002	0.526±0.03	0.334±0.01	0.629±0.03	0.535±0.02
ANT	BDL	BDL	0.037±0.01	0.039±0.01	0.025±0.02	0.038±0.02	0.025±0.01	0.042±0.002	0.009±0.03	0.048±0.02	0.049±0.19	0.049±0.04
FLT	BDL	BDL	0.003±0.01	0.002±0.00	0.001±0.08	0.028±0.02	0.006±0.02	0.069±0.002	0.024±0.01	0.069±0.03	0.039±0.17	0.079±0.04
B(a)P	BDL	BDL	0.001±0.01	0.062±0.03	0.034±0.01	0.045±0.06	0.048±0.07	0.058±0.003	0.069±0.03	0.067±0.03	0.081±0.04	0.079±0.04
D(ah)A	BDL	BDL	0.006±0.03	0.075±0.05	0.022±0.01	0.355±0.04	0.328±0.23	0.477±0.033	0.486±0.22	0.478±0.31	0.596±0.03	0.699±0.03

BDL = below detection limit; Data are represented as means ± S.D of PAHs detected in fingerlings of *C. gariepinus*

3.0 Results and Discussion

3.1 Water quality parameters

The water quality parameters measured during the 96-hour exposure are presented in Table 1, and the dissolved oxygen (DO) was in the range of 6.20 mg/L–6.24 mg/L, pH 6.67–6.76 and temperature 27.57–27.72 °C in treatment ET_0 (0.0 µg/kg) which were higher than those in ET_1 to ET_5 for both toxicants. The decreasing DO with the increasing percentage concentration of the oils is possibly due to the anaerobic decomposition of organic matter in water, resulting in the formation of noxious and toxic substances such as hydrogen sulphide and methane that have a deleterious effect on aquatic life (Harrel, 1985). Similar results were reported in the literature (Awoyinka *et al.,* 2011; Gabriel *et al*., 2007). Although Similarly, the pH level in the treatment media decreases with increasing toxicants, suggesting deposition of mucus secretion and carbonic acid or its metabolites into the media from the fish to counter the effects of the toxicant and pose lethal effects on the juveniles of *C. gariepinus* (Awoyinka *et al.,* 2011). Similar results were reported by Sunmonu and Oloyede (2006).

3.2 Mortality Effect of the Toxicants on the C. gariepinus Fingerlings

The result of percentage mortality from the Range finding test is presented in Table 1. From the result, the mortality rate of the catfishes increases with the increasing concentration of the oils and duration of exposure. Significantly increasing mortality was recorded for fingerlings of C. gariepinus with increasing concentration and time (h). From the Table, a high mortality rate ranging between 64 (≈8 fingerlings) and 100% was recorded between 24 h and 72 h for LCO at higher concentrations (ET_3–ET_5) while for the HCO, the highest was 81% (≈10). In the blank treatment (ET_0), all the fishes survived the treatment conditions; in the ET_5 (where the highest concentration of the oils was tested), 0.6 L of LCO in the 20 L treatment solution caused all the fingerlings to die while less than 20% survived in the same treatment with HCO, suggesting that crude oils are highly toxic to aquatic organisms. Results from the acute toxicity test in the same treatment tank follow the same trend as obtained in the range-finding test with significantly varied results when LCO and HCO are compared. After 96 h in treatments ET_3–ET_5, no fingerlings survived the hardship condition imposed by LCO while about 25% and 11% survived from similar treatment with HCO in ET_3 and ET_4, respectively. Light crude showed a significant difference in mortality rate ($P< 0.05$) in the studied fish compared to heavy crude oils, and this may be attributed to the very high volatility and toxicity of LCO over HCO. This corroborates findings by Fabio *et al.* (2016) on haematological, biochemical and histopathological changes from large commercial fish contaminated water with heavy metals.

A strong correlation occurs between the water quality (Table 1) and the mortality rate in Table 2 in all the treatment tanks, it was observed that mortality of C. *gariepinus* increases as the D.O, pH and temperature reduce, suggesting that the living condition of the fishes were impacted as the amount of crude oils increases in water.

3.3 Bioaccumulated PAHs in C. gariepinus

This study sought to investigate the amount of 9 PAHs *C. gariepinus* could accumulate from both LCO and HCO, and their concentrations are presented in Table 3. Generally, from the Table, the concentrations of PAHs detected in the fishes were low which could be due to the low volume of the oils used; although, they increase with the increasing percentage of crude oil in the test media. The blank sample (ET_0) presents no PAHs in the Fishes, suggesting that the borehole water used for the study did not contain PAHs as well as no PAHs are introduced during experimental and analytical processes. This low concentration of PAHs could be due to a lost volatile PAHs (especially those from the LCO) to the environment or due to the excretion of metabolic waste by the fishes. There was no special pattern in the results in terms of high and low, but they vary significantly ($P<0.05$) between LCO and HCO in all treatments except in ET_0 where no toxicant was added. Similar results were reported by Wangboje and Okpobo (2019) on the carcinogenic risk of PAHs in selected smoked fish species from a typical rural market in West Africa. There was no noticeable correlation between the volume of oil exposed, mortality and the concentration of PAHs in the dead fishes. A further study may establish this by determining the concentration of PAHs in the oils separately, a study which is out of the scope of this report.

4.0 Conclusion

Crude oil spillage introduces various chemicals that are toxic to the environment and pose different health risks to the exposed organisms, including humans. This study established that the presence of crude oils alters the environment and organisms living organisms, depending on the amount and exposure duration. It was established that crude oils, especially the light crude oil, reduce the population of *C. gariepinus* in the freshwater system and this may apply to species in other aquatic ecosystems, as well as causing problems to the entire biodiversity. Thus, there is a need for regular monitoring of oil spillage, even in small amounts, to help conserve the aquatic environment in the oil-producing areas. It is also suggested that relevant agencies sensitize the inhabitants of oil-producing areas on possible risks associated with areas contaminated with light and heavy crude, especially consumption of exposed fishes and other seafood. More researches are expedient on acute toxicity and bioremediation on light and heavy crude on other fish species and the field.

CRediT authorship contribution statement

EGI: Conceptualization, Methodology, Resources, Writing-original draft, Writing-review & editing. AUO: Methodology,

Writing - original draft, Writing-review & editing. MOS: Writing-original draft, Writing -review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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