***Chemistry of the Total Environment* 2022, 2(1): 10-16**

GLINTPLUS

ISSN: 2805-3419

Original Article

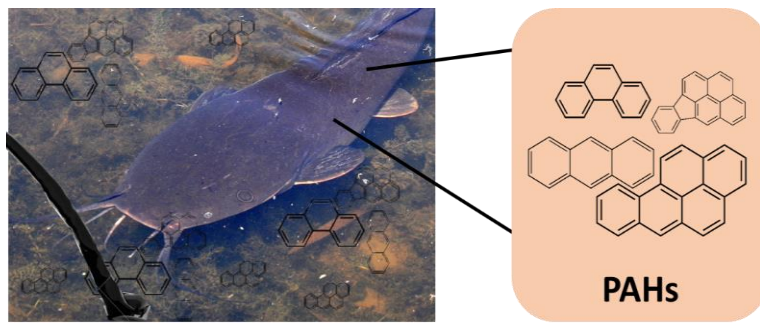
Concentrations of polycyclic aromatic hydrocarbons (PAHs) in the African catfish (*Clarias gariepinus*) juveniles exposed to crude oil contaminated water

Augustine U. Orowe[a, *], and Efe G. Ikponmwen[b]

[a] Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Benin City, Nigeria; [b] Department of Fisheries and Aquaculture, Federal University Wukari, Taraba State, Nigeria.

ABSTRACT

The concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) were investigated in the African catfish (*Clarias gariepinus*) juveniles exposed to crude oil using gas chromatography coupled with mass spectrometry. A total of 180 juvenile catfish of weight ranging from 7.5-8.3g (7.993 ±0.98g) and length 9.3-10.1cm (9.7 ± 0.72cm) were exposed to crude oil of varying concentrations 0.0% - 1.0% (labelled TA-TF, respectively) of crude oil for 480 h. The highest temperature value (25.25 °C) was recorded in the TF, while the highest level of dissolved oxygen was recorded in the TA (6.0 mg/l). pH values ranged from neutral (7.0) in the control to slightly alkaline (7.4 – 8.0) in the various treatment media. Significantly different (P<0.05) concentrations of PAHs were obtained with the highest level of PAHs (10.754 µg/kg) in fish from TF. The overall results showed that crude oil in the aquatic environment has negative effects on fish fauna. Fishes in oil-polluted water can accumulate PAHs in their flesh and organs; therefore, it is not advisable to consume fish from such a contaminated environment due to the high health risk associated. Regular investigation of oil-producing areas for environmental contaminants and prompt remediation of polluted areas by the authority are recommended.



HIGHLIGHTS

- African catfish (*Clarias gariepinus*) were exposed to crude oil contaminated water.
- Levels of PAHs contributed from crude oil were determined using GC-MS.
- The 16 USEPA priority PAHs were recorded in fishes from all the treatment setups.
- Concentration of B(a)P was below the maximum standard by ECR.

Article History:
Received: 28th October, 2021
Revised: 28th December, 2021
Accepted: 13th March, 2022
Available online: 31st March, 2022

Keywords:
Crude oil, PAHs, carcinogen, *Clarias gariepinus*.

1. Introduction

Crude oil spillage is a common problem in oil-producing areas all over the world. Several pollutants that are known to cause both direct and indirect problems to the ecosystem, humans and other organisms are released to the immediate environment. Exposure of aquatic organisms to crude and refined oils impacts various aspects of fish physiology, sometimes leading to large scale mortality (Liu *et al*., 2006). Azad (2005) observed that eggs and young stages (fingerlings) of fishes are especially vulnerable to the toxic effects of water-soluble components of crude oil and its refined products. This, according to Adeola (1996), has resulted in the decimation of the rich fisheries resources in Nigerian aquatic areas.

In Nigeria, crude oil was discovered at Oloibiri (in, present-day, Bayelsa State) in 1956, and has generated so much revenue for the country (Akpofure *et al*., 2000). However, this is not without its attendant problem of spillage into adjoining water bodies and farmlands. *Clarias gariepinus* is produced in both natural water bodies and fish ponds, and these waters are often polluted with oil spills or petroleum products. The consumption of such fishes is associated with some health risks. Each year, according to Sunmonu and Oloyede (2007), an average of 14 million gallons of crude oil from more than 10,000 accidental spills is discharged into water bodies worldwide including Nigeria, particularly through the leakage of pipes carrying oil and from underground reserves.

Crude oil is a structurally complex, heterogeneous mixture composed of simple aliphatic hydrocarbons (SAHs), including polycyclic aromatic hydrocarbons (PAHs), resins and asphaltenes (Abbriano *et al*., 2011). PAHs represent an important class of carcinogen whose presence in foods have been extensively studied. Of the several hundreds of known PAHs, sixteen have been identified as priority PAHs because

* CONTACT: A. U. Orowe; austinorowe@gmail.com; Department of Aquaculture and Fisheries Management, University of Benin, Benin City, Nigeria.
https://doi.org/10.52493/jcote.2022.2.31

they are considered more harmful to humans than the others (Chimezie and Hebert, 2006; Wretling, *et al*., 2010). The deleterious health effects of PAHs exposure to the human body have been reported in several previous studies in the world (Arowojolu *et al*., 2021). In particular, benzo[a]pyrene is the first compound that can cause cancer (Al-Thaiban *et al*., 2018). Due to the serious impact of PAHs on human health and the ecosystem, the United States of America Environmental Protection Agency (US-EPA) has introduced 16 PAHs on the list of priority organic pollutants (Manh *et al*., 2019). PAHs are toxic and can induce toxic symptoms in experimental animals (Nicolas, 1999). PAHs a class of high lipophilic compounds consist of chemical compounds known to be potent carcinogens. PAHs are ubiquitous and present in all the environmental compartments (water, air, soil, sediment), and traces of PAHs have been found in various food products (Silva, *et al*., 2011).

Due to their carcinogenic nature, PAHs have been included in the European Union (EU) and the United States Environmental Protection Agency (USEPA) priority pollutant lists. Inhalation, dermal contact and consumption of contaminated foods are the three exposure channels of PAHs to human beings, and the latter accounts for 88 to 98% of the contaminant getting into the human body (Farhadian *et al*., 2011). The consumption of PAHs-contaminated *Clarias gariepinus* may exert negative effects on man physiologically and are of public health concern. PAHs can cause histological and haematological damage to the gills, liver, heart and other organs of the exposed fish (Orowe *et al*., 2017; Orowe *et al*., 2015). A further problem is the tainting of the flesh of fish, which is detectable at very low contamination and renders fish inedible. Fish and other aquatic organisms can accumulate contaminants (including PAHs) from the environment (*Ubani et al*., 2006).

A lot of research work has been done on the concentration of PAHs in airborne particulate matter (Vu-Duc *et al*., 2021; Galmiche *et al*., 2021) in smoked meat and fish products (Al-Thaiban *et al*., 2018; Wangboje and Opobo 2019; Šimko *et al*., 2002) and on the histological and haematological effects of crude oil on juvenile *Clarias gariepinus* (Orowe et al., 2017; Orowe et al., 2015). Jack *et al*. (2005) also reported that significant levels of total hydrocarbons in shellfishes in higher crude oil polluted sites in the Niger-Delta area of Nigeria were higher when compared with unpolluted sites. However, there is scanty information on the concentration of PAHs in juvenile *Clarias gariepinus* exposed to crude oil in Nigeria. The study was aimed at determining the levels of PAHs in laboratory stored juvenile African catfish, *Clarias gariepinus* using GC-MS techniques. Also, some common physicochemical parameters that can impact the healthy living of the fish were determined

2.0 Materials and Methods

2.1 Materials and Reagents

Crude oil was gotten from the Lease Automatic Custody Transfer (LACT) unit of Nigerian Petroleum Development Company (NPDC) at Pan Ocean, Oredo Flow Station, Edo State, Nigeria and stored in a well-labelled sterilized plastic container (Sunmonu and Oloyede, 2006). Gas chromatograph (Model Agilent 6890), Vacuum rotating evaporator, Electrical weighing balance (Ohaus corporation USA Model: scout pro SPLI 401), Hanna instrument (9-series multi water quality meter), monofilament netting, experimental glass tanks (50 × 25 × 26) cm^3, glass rod, Sintered funnel, Extraction bottle with Teflon cover, refrigerator Analytical grade (Hexane and Acetone 98%), Anhydrous Sodium sulphate (99% purity), activated silica, silica gel, dichloromethane (DCM, 98%), all were purchased from Labwaco Chemical, Benin City, Nigeria.

One hundred and eighty (180) healthy live juveniles of the African catfish (*Clarias gariepinus*) were bought early in the morning from Samdoc Fish Farm, Benin City, Nigeria and stored in plastic containers transported to the lab in well-aerated oxygenated bags and acclimated to laboratory conditions with borehole water for seven days and fed 2mm Coppens feed at 4% body weight twice a day, at 8.00 am and 4.00 pm (Gabriel *et al*., 2007) before exposure test. Standard of PAHs containing naphthalene (NAP), acenaphthylene (ACN), acenaphthene (ACL), fluorine (FLR), phenanthrene (PHT), anthracene (ANT), pyrene (PYR), benzo(a)anthracene (B(a)A), chrysene (CRY), benzo(b) fluoranthene (B(b)F), fluoranthene (FLT), benzo(k)fluoranthene (B(k)F), benzo(a)pyrene (B(a)P), indeno (1,2,3-cd) pyrene (IP), dibenzo[a,h]anthracene (D(ah)A), and benzo (g,h,i)perylene (B(ghi)P)) was purchased from Labwaco Chemicals, Benin City, Nigeria to prepare GC-MS working solution.

2.2 The Exposure Test

The exposure experiment was laid out in a completely randomized design, consisting of six treatments (0.0%, control) 0.1%, 0.25%, 0.5%, 0.75%, and 1% (labelled as TA-TF, respectively) of crude oil in 25L water for 480 h in three replicates. Ten fishes were loaded into each tank, the tanks (18) were arranged in a group of three rows and the water of the system was changed every two days with the appropriate percentages of the crude oil added.

2.3 Physicochemical Analysis

The temperature and pH of the exposure setup were measured daily according to the method described elsewhere (Jingxi *et al*., 2020; APHA, 1992). Briefly, 3 known buffer solutions (pH 4, 7 and 10) were prepared and used to standardize the multi-meter. The meter was inserted into the water samples collected from the various tanks and the pH and temperature readings were taken. Dissolved oxygen was determined using azide modification of Winkler's method. Approximately 200 mL of the water sample was transferred into a 300 mL BOD bottle and 1 mL of manganese sulphate solution and 1 mL of the alkaline alkali-iodide-azide reagent were sequentially added. The mixture was then titrated against 0.025 N sodium thiosulphate with the endpoint indicated with a brownish-orange colour. The titre value was recorded as the dissolved oxygen content of the system.

2.4 PAHs Extraction

The PAHs in the fishes were extracted according to the method described in Amzad *et al*., 2014; Garcia-Falcon, 2005; Brammer and Puyear, 1982; and Keke, 1997 with slight modification. A fish (whole) from each tank was picked, killed, oven-dried and milled to powder using porcelain mortar and pestle, transferred to an extraction bottle, added 50 mL of DCM, covered and allowed to interact for about 30 min. The solution was centrifuged and filtered using a sintered funnel. The filtrate was concentrated to about 3 mL using a rotary evaporator, cleaned up in a column packed with silica gel and eluted with 30mL of DCM for PAHs. The eluents were then concentrated to about 2-3mL, transferred to 5 mL amber vials and stored at -20 °C before instrumental analysis.

2.5 PAHs Analysis

An Agilent 6890 gas chromatograph (GC) interfaced with an Agilent 5973N Mass Spectrometer (MS) was used in this study. The samples were injected into GC comprising of META X5 coated fused capillary column length: 30 m, diameter: 0.25 mm, a film thickness of 0.25 μm; and ultra-high pure helium (99.99%) as carrier gas (1 mLmin-1) through an automatic liquid sampler injector (Agilent 7683 Series) in a split mode at an injector port temperature of 300 °C. 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 retention time range for PAHs in this study 10.0 – 27.8 min is within the range of value (7.2 – 26.09 min) reported for PAHs in the airborne matter by Nam *et al*. (2021) but lower than (10.2 – 29.1 min) recorded by Ramalhosa *et al*. (2009) for horse mackerel, chub mackerel, sardine and seabass contaminated with PAHs.

2.6 Statistical analysis

A GENSTAT ® software (version 12.1 for windows) was used for the statistical analysis. Data generated were subjected to Analysis of Variance (ANOVA) to determine the significant differences between mean values of PAHs in the spiked fishes at 95% levels of significance. Significant means were separated using Duncan multiple range test (DMRT) (Duncan, 1955).

3.0 Results and Discussion

3.1 Physicochemical properties of the spiked water

The results of the physicochemical parameters measured during the 20 days exposure period are presented in Table 1. The parameters were temperature, dissolved oxygen and pH. The values of these parameters were significantly different (P<0.05) from each other. The values for pH obtained at the end of the 20 days exposure period tended towards neutral in the TA and TB. The pH values were slightly in the alkaline (7.9 – 8.0) range, and there were no significant differences (P>0.05) between the pH values of the ponds. The values for the dissolved oxygen recorded in the various treatment were significantly different from one another (P<0.05). There were no significant differences (P>0.05) between treatments TA (6.0 mg/L) and TB (5.9 mg /L) and between TD (5.0 mg/L) and TE (4.9 mg/L). Temperature values recorded in the various treatments were significantly different (P<0.05) from each other with the highest mean value (25.25 °C) obtained the TF and the lowest value (24.75 °C) obtained in the TA.

Table 1. Mean values of physicochemical parameters in water exposed to crude oil

Treatment	Temperature (°C)	Dissolved Oxygen (mg/L)	pH
TA	24.75^{f}	6.000^{a}	7.000^{a}
TB	24.80^{e}	5.900^{a}	7.400^{b}
TC	25.00^{d}	5.500^{b}	7.900^{c}
TD	25.10^{c}	5.000^{c}	8.000^{c}
TE	25.21^{b}	4.900^{c}	8.000^{c}
TF	25.25^{a}	4.500^{d}	8.000^{c}

^{abc} Means with different superscripts in the row differ significantly(P<0.05); TA-TF represent treatment units containing 0.0-1.0% of crude oil.

The results of the physical and chemical analyses of contents of the experimental tanks are essential in evaluating water quality, as they provide important data about the variations and effects on experimental fish caused by the different concentrations of crude oil. Temperature is known to have a strong influence on enzyme reaction, growth efficiency, reproduction and immune response in fish (Tanck *et al*., 2000). In this study, slightly higher temperatures were recorded in the various treatments relative to the control. Orowe and Oguzie (2015) also observed a similar increase in temperature in tanks exposed to various concentrations of crude oil. Temperature values obtained showed that the crude oil concentration slightly affected water quality. Ullrich and Millemann (1983) reported that there is a direct relationship between temperature and the sensitivity of an organism due to increased toxicant uptake at high temperature, leading to reduced food intake by the organisms. Also, Bat *et al*. (2000) reported that the mortality of *Gammarus pulex* increased with increasing copper, zinc and lead concentrations and temperature regimes. Similar effects of temperature were reported by Bryant *et al*. (1985) on the toxicity of chromium, arsenic, nickel and zinc to a variety of marine and estuarine invertebrates.

A slight increase in temperature was reported to reduce the oxygen-carrying capacity of the water and was shown to increase the sensitivity to oil by sturgeons (*Accipenser stellatus* and *Huso huso*) (Brunges *et al*., 1978). None of the concentrations in this study showed dissolved oxygen value below 4 mg/L concentration considered critical by Esteves 1988). The presence of oil in the aquatic environment in this

Table 2. Concentration of Polycyclic Aromatic Hydrocarbons (PAHs, μg/kg) in *Clarias gariepinus* Juveniles

PAHs	No. of Ring	TA	TB	TC	TD	TE	TF
NAP	2	0.0000±0.0000[e]	0.3487±0.0001[d]	0.4010±0.0001[d]	0.5430±0.0000[c]	0.6058±0.0000[b]	0.7880±0.0000[a]
ACN	3	0.0000±0.0000[d]	0.4064±0.0017[c]	0.4580±0.0017[c]	0.6000±0.0001[b]	0.6528±0.0001[a]	0.7043±0.0000[a]
ACL	3	0.0000±0.0000[c]	0.7033±0.0011[b]	0.6551±0.0001[c]	0.6971±0.0001[c]	0.7699±0.0001[b]	0.9728±0.0000[a]
FLR	3	0.0000±0.0000[e]	0.3400±0.0000[d]	0.4924±0.0001[c]	0.7344±0.0001[b]	0.7972±0.0000[b]	0.9002±0.0000[a]
PHT	3	0.0000±0.0000[e]	0.0900±0.0000[d]	0.1424±0.0000[c]	0.4511±0.0000[b]	0.5239±0.0000[a]	0.5269±0.0000[a]
ANT	3	0.0000±0.0000[f]	0.2007±0.0001[e]	0.2531±0.0004[d]	0.3751±0.0001[c]	0.4279±0.0000[b]	0.5824±0.0000[a]
FLT	4	0.0000±0.0000[f]	0.3008±0.0002[e]	0.3533±0.0002[d]	0.5153±0.0001[c]	0.5781±0.0001[b]	0.6811±0.0000[a]
PYR	4	0.0000±0.0000[f]	0.1506±0.0001[e]	0.2030±0.0002[d]	0.3450±0.0001[c]	0.3978±0.0001[b]	0.4978±0.0000[a]
B(a)A	4	0.0000±0.0000[e]	0.4001±0.0001[d]	0.4525±0.0001[d]	0.5945±0.0001[c]	0.6673±0.0000[b]	0.7733±0.0000[a]
CRY	4	0.0000±0.0000[d]	0.6027±0.0001[c]	0.5502±0.0001[c]	0.7922±0.0000[b]	0.8650±0.0000[a]	0.9165±0.0000[a]
B(b)F	5	0.0000±0.0000[e]	0.3018±0.0003[d]	0.4543±0.0002[c]	0.4963±0.0000[b]	0.5491±0.0000[b]	0.7036±0.0000[a]
B(k)F	5	0.0000±0.0000[f]	0.4009±0.0001[e]	0.4533±0.0001[d]	0.5953±0.0000[c]	0.6573±0.0000[b]	0.7603±0.0000[a]
B(a)P	5	0.0000±0.0000[b]	0.0000±0.0000[b]	0.0001±0.0000[a]	0.0000±0.0000[b]	0.0000±0.0000[b]	0.0002±0.0000[a]
D(a,h)A	5	0.0000±0.0000[b]	0.3002±0.0001[c]	0.3527±0.0000[b]	0.3669±0.0000[b]	0.3977±0.0000[b]	0.5318±0.0000[a]
B(g,h,l)P	6	0.0000±0.0000[d]	0.7017±0.0001[a]	0.5042±0.0008[c]	0.5462±0.0000[c]	0.6421±0.0000[b]	0.6357±0.0000[b]
IP	6	0.0000±0.0000[e]	0.1001±0.0001[d]	0.2049±0.0002[c]	0.6133±0.0001[b]	0.6761±0.0001[b]	0.7791±0.0000[a]
Cumulative concentration		0.00000[f]	5.3480[e]	6.1350[d]	8.2661[c]	9.2082[b]	10.7540[a]

[abc] Means with different superscripts in the column differ significantly (P<0.05); TA-TF represent treatment units containing 0.0-1.0% of crude oil.

study tends to cause a decrease in oxygen level largely due to enhanced microbial activity Scott *et al*.(1984) and Val and Almeida-Val (1999).

There was a decrease in dissolved oxygen concentration in all the treatments relative to the control. Dissolved oxygen concentration in the TF (4.5 mg/L) does not agree with the minimum DO of 5.0 mg/L reported for tropical fishes by Saloon and Scot Duncan (2005) and such might not sustain life. Orowe (2016) investigated Concentration of Polycyclic Aromatic Hydrocarbons (PAHs) and Simple Aliphatic Hydrocarbons (SAHs) in the Flesh of the *C. gariepinus* during exposure to crude oil and bioremediation effect and Mitchell and Bennett (1972) examined the toxicity of crude oil to the bluegill sunfish (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*) using a static test. Results showed that oil on the surface of the test jars prevented gaseous diffusion, leading to a build-up of carbon dioxide in the water, causing oxygen stress in channel catfish. Catfish gulped for air at the water surface and became exposed to more oil, whereas bluegills were not oxygen stressed.

Compared to the control, there were significant differences (P<0.05) between the pH of the control and crude oil concentrations, with the various concentrations tending towards neutral and slightly alkaline. Sunmonu and Oloyede (2006) reported a similar trend and suggested that this may be attributable to the deposition of carbonic acid or its metabolites into the medium accompanied by mucus secretion from the catfish into the water environment in their bid to survive.

3.2 Levels of PAHs in the exposed fishes

The results of the levels of PAHs determined in the whole fish are presented in Table 2. Generally, the concentrations of PAHs in the treatment units increase as the percentage of crude oil increases from TB to TF. The lowest mean level of PAHs (5.348 μg/kg) was recorded in fish samples from the TB while the highest was recorded in the TF. The PAHs levels are significantly different in the fishes from the media at p<0.05, suggesting that the effects of the crude oil on the species are significantly different and fishes in TF may suffer the health risks associated to PAHs than others from other treatment units. Although, there was no mortality in all the units during the period of exposure. This finding corroborated reports of Mitchell and Bennett (1972) who recorded no mortality in the different concentrations of the water-soluble fractions of crude oil used in their study on channel catfish (*Ictalurus punctatus*). The result also supported the finding of Morrow *et al*. (1975) on young coho salmon (*Oncorhynchus kisufch*), that while aliphatic compounds produced no toxic effects, aromatic compounds (PAHs) showed increased toxicity on the fish. The reduced effects of the oil on the species may be connected with the loss of volatile materials due to their experimental method, and the evaporation of volatile materials may occur in static systems so that test solutions may appear less toxic than they really would be (Hedtke and Puglisi, 1982).

The pollution of the aquatic ecosystem by crude oil spillage usually leads to stress of aquatic biota and accumulation of carcinogenic PAHs in the flesh of catfish. This may ultimately have serious consequences on humans who feed on fish. The cumulative amounts of PAHs (10.754 µg/kg) were highest in treatment TF (1.0% concentration) and lowest in treatment TB (5.348 µg/kg) and no PAHs detected in the blank samples (treatment TA; 0.0%) The data obtained from the present study showed that PAHs, particularly Benzo (a) pyrene concentration in the fish flesh was 0.0 µg/kg which is far below the maximum standard set by European Commission Regulation (ECR, 2006) for smoked (5.0µg/kg) and fresh (2.0 µg/kg) fish.

4.0 Conclusion

The observed effects of crude oil concentration on *C. gariepinus* and water quality (temperature, pH and dissolved oxygen) were related to the crude oil concentration and the accumulation of PAHs in the fish leading to damage of functional organs, the aquatic environment and human health, which are closely interrelated. The concentration of Benzo (a) pyrene (0.0 µg/kg) was below the maximum standard set by EC for fresh fish (2.0 µg/kg). Since the *Clarias gariepinus* accumulated PAHs from the crude oil polluted water in their flesh, it is recommended that fishes from such contaminated environments should not be consumed as they may introduce carcinogenic substances into the human body. Likewise, regular check-ups of the surroundings of oil-producing areas and clean-up of polluted areas are highly recommended for healthy living of the exposed biodiversity and humans.

CRediT authorship contribution statement

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

References

Abbriano, R.M., Carranza, M.M., Hogle, S.L., Levin, R.A., Netburn, A.N., Seto, K.L., Snyder, S.M. and Franks, P.J.S. (2011). Deepwater Horizon Oil Spill: A review of the planktonic response. Oceanography 24(3):294 – 301, http://dx.doi.org/10.5670/oceanog.2011.80.

Adeola, M.O. (1996). Present and potential sources of water pollution: effects on fish and wildlife resources. In: Aina, E. O. A. and Adedife, N. O. (Eds). FEPA Monograph 6: 191 – 197.

Akpofure, E.A., Efere, M.L. and Ayawei, P. (2000).Oil spillage in Nigeria's Niger Delta integrated grass root impact assessment of Acute damaging effects of continuous oil spillage in Niger Delta: A paper report on spillage in Niger Delta. Port-Harcourt, 2000. https://doi.org/10.2118/167585-ms

Al-Thaiban, H., Al-Tamimi, N. and Helaleh, M. (2018). Development of QuEChERS method for the determination of polycyclic aromatic hydrocarbons in smoked meat products using GCMS from Qatar. Journal of Analytical Methods in Chemistry, vol. 2018, Article ID 9206237, 11 pages. https://doi.org/10.1155/2018/9206237

American Public Health Association (APHA)(1992). Standard methods for the examination of water and wastewater," in Apha, WEF and AWWA, A. E. Greenberg, L. S. Clesceri, and A. D. Eaton, Eds., p. 1134, 18th edition, American Public Health Association, Washington, DC, USA, 1992. https://doi.org/10.2105/ajph.82.3.476

Amzad, H. M., Yeasmin, F., Mizanur Rahman, S.M. and Rana, S. (2014) Naphthalene, a polycyclic aromatic hydrocarbon in the fish samples from the Bangsai River of Bangladesh by gas chromatograph-mass spectrometry. Arabian Journal of Chemistry 7: 976-980 https://doi.org/10.1016/j.arabjc.2010.12.014

Arowojolu, I. M., Tongu, S. M., Itodo, A. U., Sodre, F. F., Kyenge, B. A., & Nwankwo, R. C. (2021). Investigation of sources, ecological and health risks of sedimentary polycyclic aromatic hydrocarbons in River Benue, Nigeria. Environmental technology & innovation, 22, 101457. https://doi.org/10.1016/j.eti.2021.101457

Azad, M. (2005). Toxicity of water-soluble fractions of four fuels on Metaysidopsis insularis, an indigenous tropical mysid species. Environmental Monitoring and Assessment. 104:37 – 44. https://doi.org/10.1007/s10661-005-6400-0

Bat, L., Akbulut,M.,Culha,M.,Gundogdu, A. and Satilmis , H.H.(2000).Effect of temperature on the toxicity of zinc copper and lead to the freshwater amphipod *Gammarus pulex* pulex (L.,1758). Turk. J. Zool.24 :409-415

Brammer, J.D. and Puyear, R.L. (1982). Identification and qualification of water soluble components of outboard motor exhaust and of gasoline in a North Dakota Lake, and a determination of their biological effects upon selected freshwater organism. North Dakota Water Resources Institute, Fargo. https://doi.org/10.3133/908

Brunges, W. A., Carlson, R. W., Horning, W. B., McCormick, J. H., Spehar, R. L. and Yount, J. D. (1978). Effects of pollution on freshwater fish. J. Wat. Pollut. Control Fed., 50, 1582 – 1636.

Bryant, V., Newbery, D.M., McLusky, D. S. and Campbell, R. (1985). Effect of temperature and salinity on the toxicity of nickel and zinc to two estuarine invertebrates (Corophium volutator and Macoma balthica). Mar. Ecol. Prog. Ser. 24:139 – 153. https://doi.org/10.3354/meps024139

Chimezie, A. and Herbert, C. (2006). Determination of polynuclear aromatic hydrocarbons (PAHs) in selected water bodies in the Niger Delta. African J. of Biotechnology. 5(21): 2024 – 2031.

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, *11*(1), 1-42.

Esteves, F.A. (1988). Fundamentos de limnologia . rio de Janeiro, Interciênca/FINEP.

European Commission Regulation (ECR)(2006). Setting maximum levels for certain contaminants in foodstuff. Official journal of the European Union 1881: 5 – 24.

Farhadian, A. Jinap S., Hanifah H.N., Zaidul I.S. (2011). Effects of meat preheating and wrapping on the levels of polycyclic aromatic hydrocarbons in charcoal-grilled meat. Food Chem. 141-146pp. https://doi.org/10.1016/j.foodchem.2010.05.116

Gabriel, U. U., Amakiriand, E. U., and Ezeri, G. N. O. (2007) hematology and Gill Pathology of *Clarias gariepinus* Exposed to Refined Petroleum Oil, Kerosene under Laboratory Conditions. Journal of Animal and veterinary advances 6(3): 461 -465.

Galmiche, M., Delhomme, O., François, Y. N., & Millet, M. (2021). Environmental analysis of polar and non-polar polycyclic aromatic compounds in airborne particulate matter, settled dust and soot: part II: instrumental analysis and occurrence. *TrAC Trends in Analytical Chemistry*, *134*, 116146. https://doi.org/10.1016/j.trac.2020.116146

Garcia-Falcon, M.S. and Simal-Gandara, J.(2005).Polycyclic aromatic hydrocarbons in smoke from different wood and their transfer during traditional smoking into chorizo sausages with collagen and tripe casings .Food Addit. Contam. ,22 :1-8. https://doi.org/10.1080/02652030400023119

Hedtke, S.F. and Puglisi, F.A. (1982). Short-ter toxicity of five oils to four freshwater species .Arch, Environ.Contam.Toxicol.,11: 425-430. https://doi.org/10.1007/bf01056068

Jack, I. R., Fekarurhobo, G. K., Igwe, F. U. and Okorosaye-Orobite, K. (2005). Determination of total hydrocarbonslevel in some marine organization from some towns within the Rivers State of Nigeria. Journal of AppliedScience and Environmental Management. 9(3): 59-61. https://doi.org/10.4314/jasem.v9i3.17353

Jingxi, M., Shuqing, W., Ravi-Shekhar, N. V., Biswas, S. and AnoopKumar, S.(2020).Determination of Physicochemical Parameters and Levels of Heavy Metals in Food Waste Water with Environmental Effects. Bioinorganic Chemistry and Applications. Volume 2020, Article ID 8886093, 9 pages. https://doi.org/10.1155/2020/8886093

Keke, I.R. (1997). Laboratory uptake of petroleum hydrocarbons by fresh water fishes from the Niger Delta, Nigeria. Nigerian Journal of Biotechnology, 6: 190-197.

Liu, B. R., Romaire, P. D., Elaune, R. D.and Lindau, C. W. (2006). Field investigation on the toxicity of Alaska North Slope crude oil and dispersed ANSC elude to Gulf Killifish, Eastern Oyster and White Shrimp. Chem. 62:520 – 526. https://doi.org/10.1016/j.chemosphere.2005.06.054

Manh, T.T., Quoc, H.A. and Takahashi, S.(2019) “Analysis and evaluation of contamination status of PAHs in settled house and road dust samples from Hanoi,” VNU Journal of Science: Natural Sciences and Technology. 35: 4, 63–71. https://doi.org/10.25073/2588-1140/vnunst.4943

Mitchell, D. M. and Bennett, H. J. (1972). The susceptibility of bluegillsun-fish, *Lepomis* macrochirus, and channelcatfish, Ictalurus punctatus, to emulsifiers and crude oil.Proc. Louisiana Acad. Sci., 35: 20 - 26.

Morrow, J. E., Geritz, R. L., and Kirton, M. P. (1975). Effects of some components of crude oilon young cohosalmon. Copeia,75:326 - 333. https://doi.org/10.2307/1442886

Nicolas, J.M. (1999). Vitellogenesis in fish and the effects of polycyclic aromatic hydrocarbon contaminants. Aquat.Toxicol., 45:77-90. Sunmonu, T. O. and Oloyede, O. B., (2006). Changes in Liver Enzyme Activities in African Catfish (Clariasgariepinus) exposed to Crude Oil. Asian Fisheries Science 19(2006):107-112. https://doi.org/10.33997/j.afs.2006.19.2.002

Orowe, A.U (2016). Bioremediation Effect with Aqueous Moringa oleifera Leaf Extract on the Growth and Reproduction of *Clarias gariepinus* Recovered from Crude Oil Polluted Water (2016). Ph.D Thesis presented to School of Post Graduate Studies, University of Benin, Benin City

Orowe, A.U and Oguzie, F.A. (2015). The concentrations of Polycyclic Aromatic Hydrocarbons (PAHs) and Simple Aliphatic Hydrocarbons(SAHs) in the African Catfish (*Clarias gariepinus*) juveniles exposed to crude oil. Book of proceedings, University of Benin Annual Research Day (UBARD) Conference. pp 407-411.

Orowe, A.U., Oguzie, F.A. and Ikponmwen, E. G. (2017). Histological Changes in the Gills of Juvenile African Catfish (*Clarias gariepinus*) Exposed to Crude Oil. Nigerian Journal of Fisheries 14: 1&2.1147-1152

Orowe, A.U., Oguzie, F.A. and Sado, O. M. (2015). Haematological changes in Juvenile African Catfish (*Clarias gariepinus*) Exposed to Crude Oil. Journal of Agriculture, Forestry and Fisheries.14:14-17

Saloom, M. E., & Scot Duncan, R. (2005). Low dissolved oxygen levels reduce anti-predation behaviours of the freshwater clam Corbicula fluminea. *Freshwater Biology*, *50*(7), 1233-1238. https://doi.org/10.1111/j.1365-2427.2005.01396.x

Scott, B.F., Nagay, E., Dutka, B.J., Sherry, J.P., Taylor, W.D., Glooschenko, V., Wade, P.J. & Hart, J. (1984a). The fate and impact of oil and oil-dispersant mixtures in freshwater pond ecosystems: Introduction. Sci. Total Environ., 35:105 – 113. https://doi.org/10.1016/0048-9697(84)90057-3

Silva, B.O., Adetunde, O.T., Oluseyi, T.O., Olayinka, K.O. and Alo, B. I. (2011). Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. African Journal of Food Science, 5(7): 384-391.

Šimko, P. (2002). Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavouring food additives. B: Analytical Technologies in the Biomedical and life Sciences, J Chromatogra.,777: 3-18. https://doi.org/10.1016/s0378-4347(01)00438-8

Sunmonu, T. O. and Oloyede, O. B., (2006). Changes in Liver Enzyme Activities in African Catfish (*Clarias gariepinus*) exposed to Crude Oil. Asian Fisheries Science19:107 – 112. https://doi.org/10.33997/j.afs.2006.19.2.002

Sunmonu, T. O., & Oloyede, O. B. (2007). Biochemical assessment of the effects of crude oil contaminated catfish (Clarias gariepinus) on the hepatocytes and performance of rat. *African Journal of Biochemistry*

Research, *1*(5), 083-089. https://doi.org/10.33997/j.afs.2006.19.2.002

Tanck, M. W. T., Booms, G. H. R., Eding, E. H., Bonga, S. W., & Komen, J. (2000). Cold shocks: a stressor for common carp. *Journal of fish Biology*, *57*(4), 881-894. https://doi.org/10.1111/j.1095-8649.2000.tb02199.x

Ubani, C. S., Onwurah, I. N. E. and Allumamah, E. (2006). Octanol/Water partition coefficient and bioaccumulation index of bonny light crude oil in cat fish Clarias agboyiensis in laboratory-dosed sediments. Animal Research International (2006) 3(1): 422 – 425. https://doi.org/10.4314/ari.v3i1.40763

Ullrich, S.O. Jr. & Milleann, R.E. (1983). Survival, respiration, and food assimilation of Dapnia magna exposed to petroleum and coal-derived oils at three temperatures. Can. J. Fish. Aquat. Sci.,40: 17 – 26. https://doi.org/10.1139/f83-004

Val, A.L. and Almeida-Val, V.M.F. (1999). Effects of crude oil on respiratory aspects of some fish species of the Amazon. In: Val, A. L., Almeida-Val, V.M.F. (Eds.), Biology of Tropical Fish, INPA, Manaus Basil, 277 – 291pp. https://doi.org/10.1111/jfb.12896

Vu-Duc, N., Phung Thi, L. A., Le-Minh, T., Nguyen, L. A., Nguyen-Thi, H., Pham-Thi, L. H., ... & Chu, D. B. (2021). Analysis of polycyclic aromatic hydrocarbon in airborne particulate matter samples by gas chromatography in combination with tandem mass spectrometry (GC-MS/MS). *Journal of analytical methods in chemistry*, 2021. https://doi.org/10.1155/2021/6641326

Wangboje, O.M. and Opobo, J.(2019). Potential Carcinogenic Risk from Hydrocarbon in Selected smoke fish species from a typical market in West Africa, IJRRAS, 411(41): 1-10.

Wretling, S., Eriksson, A., Eskhultb, G.A., & Larson, B. (2010). Polycyclic aromatic hydrocarbons in Swedish smoked meats and fish. J. of Food Composition and Analysis,23(1): 264 – 272. https://doi.org/10.1016/j.jfca.2009.10.003