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Assessing Uptake Kinetics and Depuration Potential of Polycyclic Aromatic Hydrocarbons of Bonny Light Crude Oil on Clarias Gariepinus

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Abstract
This study assessed the uptake kinetics of polycyclic aromatic hydrocarbons of Bonny Light Crude Oil (JL/01/BLCO) in African catfish juveniles and their ability to depurate when subsequently exposed to clean water. The Catfish Juveniles were about 3.50±0.5cm in length and 0.55±0.05g in weight obtained from Aqua-Green culture centre Onne, Rivers State, and acclimated for 10-12 days. Acclimated test species were exposed to varying concentrations of the test substance in sediment and a reference toxicant for 24-96 hours. The 96hr LC_{50} of JL-01-BLCO as obtained from the sediment toxicity test was 123,596mg/kg. The sediment was spiked to achieve a sediment concentration of 1235.96mg/kg of JL-01-BLCO which is 1% of the 96hr LC_{50} and contains 15.3mg/kg PAH for the bioaccumulation test. The use of this concentration is to ensure that the test organisms remain alive all through the uptake phase of the bioaccumulation test. The Bioaccumulation phase lasted for 56 days (Uptake-28 days & Depuration-28 days) and results obtained showed that uptake and depuration of PAH occurred in the test specie at rate constants, k_1 and k_2 of 6.261kg sediment/kg fish d^{-1} and 1.146 d^{-1} respectively. The kinetic biota-sediment accumulation factor ($BSAF_k$) and steady state biota-sediment accumulation factor ($BSAF_{ss}$) were 5.00 and 5.488, based on the $MOSAIC_{bioacc}$ model ranking scheme, implying non-bio accumulative in C. gariepinus. This study further revealed that PAHs can be eliminated from fish by transferring them into clean water though this process of natural depuration is slow. Thus, catfish has shown to be good accumulators of PAHs but can be depurated if allowed to swim in clean water.
Keywords: *Bonny light crude oil, Catfish juveniles, Exposure, Uptake, Depuration*

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1. Introduction

The occurrence of crude oil spillages in Nigeria is not without deleterious impacts on both the aquatic milieu and terrestrial ecosystems (Wegwu *et al*., 2010). According to Wegwu and Akaninwor (2006), oil spills are regular features of the oil-producing communities in the NigerDelta area of Nigeria and when these spills occur, either onshore or near shore, it inevitably affects the marine and soil ecosystems. Indeed, these ecosystems are prime factors in agricultural productivity and major livelihood in the oil producing coastal areas of Nigeria (Osuji and Adesiyan, 2005). Most industrial pollutants discharged into the environment contain organic and inorganic pollutants in dissolved, suspended and insoluble forms (Adeolu *et al.*, 2016; Sawyerr *et al*., 2017). Effluents discharged into the water bodies may affect fish and other aquatic organisms, either directly or indirectly. Polycyclic aromatic hydrocarbons (PAHs), a chemical group that has two or more condensed aromatic rings, are ubiquitous compounds in air, water, and soil (Honda *et al*., 2007; Nakata *et al*., 2014), and categorized as general environmental harmful pollutants. PAHs are especially widely detected in the aquatic environment, including water, sediment, fish, benthic invertebrates, sea birds, and sea mammals (Chizhova *et al*., 2013; Honda *et al*., 2018; Hu *et al*., 2009). They accumulate in sediments and biota that are unable to efficiently eliminate them (Meador *et al*., 1995), they are classified as Persistent Organic Pollutants (POPs), bioaccumulative and toxic (PBTs-persistent, bioaccumulative and toxic chemicals with time,

they are found to be major culprit of mutagenesis and carcinogenesis (Irwin, 1997), making them compounds of great concern. Certain amount of Polycyclic Aromatic Hydrocarbon components of petroleum has the potential to bio-accumulate within susceptible aquatic organisms and by pass, by tropic transfer to other levels of the food chain (Eisler, 1987). In Nigeria, particularly in the Niger-Delta area, the discharge of crude oil into aquatic environment and its consequent pollution hazard is increasingly becoming a phenomenon of concern (Antai and Mgborno, 1993). Such episodes have created devastating socio-economic problems and health hazard to communities affected, and have been the subject of various litigation between the host community and the oil prospecting companies.

With fish constituting an important link in the food chain, its contamination by hydrocarbons causes a direct threat, not only to the entire aquatic environment, but also to humans that utilize it as food. One of such fish species that is of paramount concern due to its economic relevance is African catfish (*Clarias gariepinus*). They are of the genus *Clarias*, a highly esteemed group of fishes in tropical Africa and they command high market value (Ugwu *et al*., 2011). This study was carried out to assess the uptake kinetics of Polycyclic Aromatic Hydrocarbons of Bonny Light Crude Oil (JL/01/BLCO), on *Clarias Gariepinus* juveniles and their ability to depurate during subsequent exposure to clean water.

2. Materials and methods

2.1 Collection and storage of test organism

Juveniles of *Clarias gariepinus* (3-6 months, 250-400 mg) that have not shown signs of sexual differentiation was used in the test. The test organisms were obtained from Aqua-Green culture centre in Onne, Rivers State. Selected samples were subjected to a TPH and PAH test to determine the baseline before using it for the test, and the baseline analysis showed no contaminants in the fish, during acclimation, with less than 0.2% mortality. The Juveniles were about 3.50 ± 0.5cm in length and 0.55 ± 0.05g in weight, with lipid content of 1.04±0.02%. This specie was selected for the following reasons:

a. They are known sediment-dwellers.
b. They are easy to maintain in the laboratory.
c. Considered as one of the most important tropical catfish species for aquaculture
d. They are of "least concern (LC)" according to the IUCN red list and as such, can be used for research purposes to ascertain pollutant effects on aquatic species.

On receipt at the laboratory, the test organisms were transferred to the flow-through aquaculture system and acclimated for 10-12 days with constant aeration. They were fed on 3% bulk weight of fish food throughout the acclimation period.

2.2 Overlying water

Overlying water (freshwater) used for the test was prepared in the laboratory using analytical grade salts as described by Okoroafor, 2011. The water was synthesized in the laboratory, then its baseline physico-chemistry (pH: 7.02, Hardness: 55.8mg/l, Alkalinity: 40mg/l, Ammonia: 3.86mg/l, and PAHs: <0.001mg/l) was established before using it for the definitive test.

2.3 Collection of pristine sediment

Since this research is on sediment associated contaminants, pristine sediment is needed and which was collected from the field. Freshwater sediment used for sediment toxicity and bioaccumulation tests was collected from a relatively pristine area of the new Calabar River, Rivers State. On arrival at the laboratory a sample was taken immediately for Physico-Chemical characterization of their particle size distribution, percent moisture, percent total organic and the rest stored at 22±2℃. The physico-chemical characteristics of the freshwater sediment is as shown on Table 1. The Moisture Content of the sediment is 58.2%. This means that 100g of wet sediment contains 58.2g of water and 42.8g of dry sediment. Therefore, to achieve 100g dry weight of sediment, 233.64g of wet sediment is needed.

Table 1: Weights of sediment and volume of JL-01-BLCO used in preparing the test concentration

Nominal Concentration (mg/kg)	Dry weight of Sediment for Test (g)	Wet weight of Sediment Used (g)	Volume of Test Substance Used (µl)	Volume of Overlying Water Used (ml)
Control	100	233.6	0.00	700
123.6	100	233.6	12.40	700

2.4 Test substance (Bonny light crude oil)

The Test substance is Bonny light crude oil coded JL-01-BLCO. The test sample was duly assigned a lab number, logged into the sample register, and stored at 22+2℃ until the acute sediment toxicity and bioaccumulation studies. The test substance has a density of 0.91g/ml as determined in the laboratory using a hydrometer. The density of JL-01-BLCO was 0.91g/ml. This means that 0.91g of the test substance is equivalent to 1ml of test substance. Therefore, to achieve 1g of test substance, 1.099ml was dispensed. The treated sediment was then properly homogenized and transferred into the test chambers (1,400ml capacity) in triplicates. Overlying water was gently added from the reservoir tank through a narrow tube to avoid sediment disturbance. The final ratio of sediment to water was 1:3. Thereafter, the medium was aerated for about 20min and then allowed to stabilize for 24 hours. Since destructive sampling technique was adopted for the sediment bioaccumulation test, the test medium was prepared to cover for each of the harvest days during both the uptake and depuration phases, all in triplicates. Test organisms were introduced at the end of the sediment stabilization period into each of these test chambers. Ten (10) individuals were added per test chamber.

2.5 Bioaccumulation test methods

The bioaccumulation test comprised of a 28-day uptake phase and a 28-day depuration phase. As recommended in the referenced test protocol (EGASPIN, 2018). One percent of the LC_{50} determined from a 96hr sediment toxicity test was used for the bioaccumulation test. The use of this concentration is to ensure that the test organisms remain alive all through the uptake phase of the bioaccumulation test.

2.6 Preparation of test medium

The 96hr LC_{50} of JL-01-BLCO as obtained from the sediment toxicity test was 123,596mg/kg. For the bioaccumulation test, the sediment was spiked to achieve a sediment concentration of 1235.96mg/kg of JL-01-BLCO, being one percent (1% of the LC_{50} and contains 15.3mg/kg PAH). The calculations are as described below.

2.7 Uptake phase

The uptake phase lasted for 28 days. Fish, sediment, and overlying water samples were taken from the allotted test chambers in appropriate sampling containers on days 1, 3, 7, 21 and 28. These were then preserved by cooling to <4ºC before transfer to the testing laboratory for quantification of polycyclic aromatic hydrocarbons (PAH) in each of the samples. The physico-chemical quality of the overlying water was constantly monitored in the test chambers throughout the uptake phase. This was done to ensure that pH, temperature, dissolved oxygen (DO) levels and other relevant parameters were within range for survival of the test organisms. Overlying water was carefully syphoned and replaced every 2-3 days during the uptake phase. This is because the toxicant was homogenized with the sediment. And since the organism is a sediment dweller, it is expected that it would interact more with the sediment than overlying water and as such, the bioaccumulation process will not be compromised. Growth, mortality, and behaviour such as sediment avoidance, erratic movement and other signs of stress were also monitored in each of the test chambers.

2.8 Depuration phase

After the 28th day of the uptake phase, the test organisms in the spiked sediment test chambers allotted for the depuration phase were transferred to another set of test chambers containing only clean overlying/habitat water. Each chamber was properly labelled according to the expected harvest/sampling days and then monitored for the 28-day depuration phase. Fish, sediment, and habitat water samples were taken from the allotted test chambers on day 1, 3, 7, 21 and 28. These were preserved by cooling to <4ºC before transfer to the testing laboratory for quantification of polycyclic aromatic hydrocarbons (PAH) in each of the samples. Similar to the uptake phase, physicochemical and biological parameters were monitored all through the depuration phase.

2.9 $MOSAIC_{bioacc}$ Model

The summary of the data used are stated below.
File used: Clarias_gariepinus_single_up-depur.csv
Exposure: 15.3 $\mu g.\ g^{-1}$
Accumulation phase duration: 56 days
Number of replicates: 3
Times : 0, 1, 3, 7, 21, 28, 29, 31, 35, 49, 56.
Exposure routes : sediment
Elimination routes : excretion
Three MCMC chains were used to estimate model parameters.
Number of iterations: 146094 Thin: 39

2.10 TK model

The TK model used for these calculations was;

$$\frac{dC_p(t)}{dt} = k_{us} \text{ x } c_s - (k_{ee}) \text{ x } C_p(t) \text{ for } 0 \leq t \geq t_c \quad (1)$$

$$\frac{dC_p(t)}{dt} = - (k_{ee}) \text{ x } C_p(t) \text{ for } t > t_c \quad (2)$$

t: Time (expressed in days)

t_c: Duration of the accumulation phase (expressed in days)

C_p(t): Internal concentration of the parent compound at time (expressed in $\mu g.g^{-1}$)

k_{ee}: Elimination rates of excretion (expressed per day^{-1})

C_s: Exposure concentration of sediment route (expressed in $\mu g.\ g^{-1}$)

k_{us}: Uptake rate of sediment exposure (expressed per day^{-1})

2.11 Bioaccumulation factor calculation

The kinetic biota-sediment accumulation factor ($BSAF_k$) and steady state biota-sediment accumulation factor ($BSAF_{ss}$) were calculated using Equations (3) and (4), respectively.

$$BSAF_k = \frac{k_{us}}{k_{ee}} \quad (3)$$

$$BSAF_{ss} = \frac{C_p(t)}{C_s} \quad (4)$$

2.12 General linear model for bioaccumulation

Based on the estimates generated from the Mosaic Bioacc Model. The estimates for uptake rate, depuration rate and bioaccumulation rates were derived from the Mosaic model. A simple linear model was created using multilinear stepwise regression, which is a step-by-step iterative construction of a regression model that involves the selection of independent variables to be used in a final model. The models created were a function of time, concentration. The models were generated using the Stata software. With 1000 Number of iterations. The biota-sediment accumulation factor (BSAF) was calculated using Equation (5).

$$BSAF = \frac{C_0/f_t}{C_s/f_{soc}} \quad (5)$$

Where C_o is the chemical concentration in organism (µg/kg ww), f_t is the lipid fraction of the organism (g lipid/g wet weight), C_s is the chemical concentration in sediment (µg/kg dry wet), and f_{soc} is the fraction of sediment as organic carbon (g organic carbon/g dry weight). The specific growth rate (Lugert *et al.*, 2016) in the test and control chambers were determined using Equation (6).

$$Specific\ growth\ rate\ (SGR) = \frac{MFW\ (Mean\ final\ weight) - MIW\ (Mean\ initial\ weight) x\ 100}{Time\ in\ days} \quad (6)$$

3. Results

3.1 Specific growth rate

The Specific growth rate (SGR) expressed in terms of weight and length of the test specie during the bioaccumulation study for exposed and control organisms are shown in Table 2. The recorded data on observed growth (length and weight) of the test species during uptake and control are presented in Tables 3-6. The uptake specific growth rate (length) of the test specie was 8.68cm/day for exposed organisms and 11.57cm/day for the control while 2.39g/day and 6.14g/day represent SGR (weight) for exposed and controlled organisms respectively. SGR in the controlled setup for both length and weight were much higher compared to the uptake. In like manner, SGR reported during depuration and control for length and weight were 6.96cm/day and 7.54g/day for exposed organisms whereas 15.18cm/day and 11.71g/day were obtained as length and weight for the control.

Table 2: Specific growth rate of test species during the bioaccumulation study

Test Phase	Parameter	Growth Rate	
		Exposed Organisms	Control Organisms
Uptake	Length	8.68	11.57
Depuration	Length	6.96	15.18
Uptake	Weight	2.39	6.14
Depuration	Weight	7.54	11.71

Table 3: Length (cm) of test species exposed to test substance during the study

Test Phase	Harvest Day	Range	Mean	SD	CV
Uptake	Day 1	2.01-2.72	2.37	0.34	0.14
	Day 3	2.48-2.95	2.73	0.19	0.07
	Day 7	2.95-3.32	3.13	0.18	0.06

	Day 21	4.01-5.15	4.30	0.48	0.11
	Day 28	3.90-5.25	4.80	0.54	0.11
Depuration	Day 1	4.00-5.90	4.85	0.75	0.16
	Day 3	4.25-5.60	4.89	0.55	0.11
	Day 7	4.20-5.20	4.86	0.40	0.08
	Day 21	5.90-6.90	6.47	0.51	0.08
	Day 28	5.20-8.80	6.80	1.49	0.22

Table 4: Length of test species in the control chamber during the study

Test Phase	Harvest Day	Range	Mean	SD	CV
Uptake	Day 1	2.35-2.40	2.36	0.02	0.01
	Day 3	2.56-2.96	2.73	0.15	0.05
	Day 7	2.85-3.89	3.36	0.45	0.13
	Day 21	4.01-5.62	5.07	0.62	0.12
	Day 28	5.52-5.65	5.60	0.05	0.01
Depuration	Day 1	5.52-5.65	5.60	0.05	0.01
	Day 3	5.65-5.85	5.77	0.08	0.01
	Day 7	5.89-6.08	6.01	0.08	0.01
	Day 21	7.42-9.70	8.68	0.95	0.11
	Day 28	9.7-10.13	9.85	0.17	0.02

Table 5: Weight gain (grams) of test species exposed to test substance during the study

Test Phase	Harvest Day	Range	Mean	SD	CV
Uptake	Day 1	0.20-0.32	0.26	0.05	0.19
	Day 3	0.33-0.46	0.41	0.06	0.14
	Day 7	0.54-0.89	0.65	0.14	0.22
	Day 21	0.73-1.04	0.92	0.14	0.15
	Day 28	0.65-1.25	0.93	0.26	0.28
Depuration	Day 1	0.49-1.59	0.91	0.45	0.49
	Day 3	0.65-1.33	0.97	0.26	0.27
	Day 7	0.77-1.12	0.99	0.14	0.14
	Day 21	1.68-2.71	2.29	0.54	0.24
	Day 28	1.32-6.44	3.02	2.16	0.71

Table 6: Weight gain (grams) of test species in the control chamber during the study

Test Phase	Harvest Day	Range	Mean	SD	CV
Uptake	Day 1	0.30-0.38	0.35	0.04	0.10
	Day 3	0.36-0.41	0.39	0.02	0.05
	Day 7	0.59-1.02	0.84	0.18	0.21
	Day 21	1.77-2.03	1.87	0.12	0.07
	Day 28	1.93-2.22	2.07	0.11	0.05
Depuration	Day 1	2.03-2.43	2.18	0.15	0.07
	Day 3	2.09-2.49	2.22	0.15	0.07
	Day 7	2.15-2.52	2.29	0.14	0.06
	Day 21	3.24-6.40	4.98	1.17	0.23
	Day 28	4.86-6.65	5.46	0.70	0.13

3.2 Physicochemical characteristics of sediment and overlying water

The Polycyclic Aromatic hydrocarbon (PAH) and physicochemical parameters of the sediment and the overlying water were evaluated in line with test protocol to determine the uptake and depuration potentials of PAHs of the test substance by *C. gariepinus*. Samples of the test species, sediment and overlying water were taken on set days of 1, 3, 7, 21 and 28, respectively and the PAH content quantified by Gas chromatography. The physicochemical characteristics of overlying water is presented in Table 7. During the uptake phase, the measured physicochemical parameters ranged thus; pH (6.40 - 7.80), Temperature (17.9 - 23.2) ℃, EC (2.60 - 830) µs/cm, salinity (0.13 - 7.80) ppt, DO (4.8 - 8.8) mg/l/. For depuration, the pH (7.60 - 8.60), Temperature (16.5 - 21.8) ℃, EC (344 - 467) µs/cm, Salinity (0.18 - 8.60) ppt, DO (10.1 - 16.9) mg/l for the fresh water medium. PAH concentrations in the fish, sediment and overlying water media during the uptake and depuration phase are reported in Tables 8– 10. The mean PAH concentrations ranged from 0.11 - 0.26mg/kg in the sediment during the uptake phase and 0.17 – 0.19mg/kg in the control. Mean PAH content in the water media during the uptake phase was found to be between 0.08 – 0.13mg/l and (< 0.001) in the control. Likewise, the PAH concentrations in the water media ranges from (0.08 - 0.13) mg/l during depuration and (< 0.001) in the control. PAH levels were evidently present in both overlying water and sediment and none in the control, however, PAH concentration in both water and sediment were highest at the inception or uptake and subsequently lowered particularly in sediment due to leaching of BLCO into the overlying water. The range and mean were derived from triplicate sampling design.

Table 7: Physicochemical characteristics of habitat water

Phase	Parameter	Range	Mean	SD	CV
Uptake	pH	6.40 - 7.80	7.18	0.57	0.08
	Temp (˚C)	17.9 - 23.2	20.40	1.83	0.09
	EC (µs/cm)	260 – 830	455.2	201.8	0.44
	Salinity (ppt)	0.13 - 7.80	0.25	0.11	0.45
	DO (mg/l)	4.80 - 8.80	6.28	1.54	0.25
Depuration	pH	7.80 - 8.60	8.15	0.31	0.04
	Temp (˚C)	16.5 - 21.8	19.2	2.03	0.11
	EC (µs/cm)	344 – 467	395.8	52.3	0.13
	Salinity (ppt)	0.18 - 8.60	0.21	0.03	0.14
	DO (mg/l)	10.1 - 16.9	12.7	2.80	0.22

Table 8: PAH concentration (mg/kg) in the sediment during the uptake phase

Phase	Harvest Day	Range	Mean	SD	CV	P-value
Uptake	Control	0.17-0.19	0.18^{a}	0.01	0.06	<0.0001
	Day 1	0.25-0.26	0.26^{b}	0.01	0.02	
	Day 3	0.22-0.24	0.23^{b}	0.01	0.04	
	Day 7	0.19-0.19	0.19^{a}	0.00	0.00	
	Day 21	0.15-0.17	0.16^{b}	0.01	0.06	
	Day 28	0.11-0.11	0.11^{b}	0.00	0.00	

Note: Any mean value whose subscript is different from that of the control is significantly different from the control ($p<0.05$)

Table 9: PAH concentration (mg/l) in the water media during the uptake phase

Phase	Harvest Day	Range	Mean	SD	CV	P-value
Uptake	Control	<0.001 - <0.001	$<0.001^{\mathbf{a}}$	0.00	0.00	<0.0001
	Day 1	0.079 - 0.081	$0.08^{\mathbf{b}}$	0.001	0.01	
	Day 3	0.089 - 0.091	$0.09^{\mathbf{b}}$	0.001	0.01	
	Day 7	0.10 - 0.110	$0.10^{\mathbf{b}}$	0.006	0.06	
	Day 21	0.119 - 0.122	$0.12^{\mathbf{b}}$	0.002	0.01	
	Day 28	0.129 - 0.132	$0.13^{\mathbf{b}}$	0.002	0.01	

Note: Any mean value whose subscript is different from that of the control is significantly different from the control ($p<0.05$)

Table 10: PAH concentration (mg/l) in the water media during the depuration phase

Phase	Harvest Day	Range	Mean	SD	CV	P-value
Depuration	Control	<0.001 - <0.001	$<0.001^{\mathbf{a}}$	0.000	0.00	<0.0001
	Day 1	0.130 - 0.131	$0.13^{\mathbf{b}}$	0.001	0.00	
	Day 3	0.099 - 0.103	$0.10^{\mathbf{b}}$	0.002	0.02	
	Day 7	0.069 - 0.072	$0.07^{\mathbf{b}}$	0.002	0.02	
	Day 21	0.049 - 0.052	$0.05^{\mathbf{b}}$	0.002	0.03	
	Day 28	0.079 - 0.082	$0.08^{\mathbf{b}}$	0.002	0.02	

Note: Any mean value whose subscript is different from that of the control is significantly different from the control ($p<0.05$)

3.3 Evaluating uptake and depuration rates (K_1 & K_2) under PAH optimum condition

Tables 11 and 12 provide a synoptic representation of uptake and depuration of PAHs that occurred in the test specie (*C. gariepinus*) subjected to the bioaccumulation test. In each harvest day, one sample each was collected from the individual three replicates and analyzed. The results were presented in ranges for each case. Mean values recorded for the respective uptake and depuration harvest days were between (0.56 – 1.04) mg/kg with the control having <0.001mg/kg. Uptake rate between day-1 and day-3 was most significant (62%) in test specie as day-7 to day-21 recorded least uptake rate (2%). Conversely, the PAH contents in the test fish depurated faster between day-7 and 21 (44%) and least from day-1 to day-3 (10%). The rate constants, K_1 and K_2 were found to be 6.261 kg sediment/kg fish d^{-1} and 1.146 d^{-1} respectively as shown in Table 13.

Table 11: PAH concentration (mg/kg) in fish sample during the uptake phase

Phase	Harvest Day	Range	Mean	SD	CV	P- value
Uptake	Control	<0.01-<0.01	$<0.01^{\mathbf{a}}$	0	0	<0.001
	Day 1	0.55-0.57	$0.56^{\mathbf{b}}$	0.01	0.02	
	Day 3	0.84-0.88	$0.86^{\mathbf{b}}$	0.02	0.02	
	Day 7	0.90-0.94	$0.92^{\mathbf{b}}$	0.02	0.02	
	Day 21	0.93-0.93	$0.93^{\mathbf{b}}$	0.00	0.00	
	Day 28	1.03-1.06	$1.04^{\mathbf{b}}$	0.02	0.01	

Note: Any mean value whose subscript is different from that of the control is significantly different from the control ($p<0.05$)

Table 12: PAH concentration (mg/kg) in fish sample during the depuration phase

Phase	Harvest Day	Range	Mean	SD	CV	P-Value
Depuration	Control	<0.01-<0.01	<0.01 **a**	0	0	<0.001
	Day 1	1.04-1.05	1.04 **b**	0.01	0.01	
	Day 2	0.98-1.00	0.99 **b**	0.01	0.01	
	Day 7	0.90-0.92	0.91 **b**	0.01	0.01	
	Day 21	0.69-0.71	0.70 **b**	0.01	0.01	
	Day 28	0.55-0.57	0.56 **b**	0.01	0.02	

Note: Any mean value whose subscript is different from that of the control is significantly different from the control ($p<0.05$)

Table 13: Uptake and depuration rate constant

Rate Constants	Values
Uptake rate (K_1)	6.261 kg sediment/kg fish d^{-1}
Depuration rate (K_2)	1.146 d^{-1}

3.4 PAHs bioaccumulation factor on *clarias gariepinus*

Bioaccumulation factor on test specie subjected to sediment spiked with BLCO were assessed using bioaccumulation model ($MOSAIC_{bioacc}$). Based on primary data generated through the bioaccumulation protocols as discussed earlier, the biota-sediment accumulation factor (BSAF) was modelled using $MOSAIC_{bioacc}$. Kinetic biota-sediment accumulation factor ($BSAF_k$) was reported to be 5.00 and steady state biota-sediment accumulation factor ($BSAF_{SS}$) was 5.488. Based on the ranking scheme, the PAH component of the test substance, JL-01-BLCO is "non-bioaccumulative" in *C. gariepinus* since the BSAF<1000. The kinetic and steady state biota-sediment accumulation factors as obtained are shown in Table 14 while the ranking scheme for chemical bioaccumulative properties is presented in Table 15. The fish and sediment concentrations used were lipid and TOC-normalized as shown in Table 16.

Table 14: Biota-sediment accumulation factor for C. Gariepinus

	2.5%	50%	97.5%	CV
Kinetic biota-sediment accumulation factor (BSAFk)	5.00	5.00	6.00	5e-02
Steady State biota-sediment accumulation factor (BSAFss)	3.5114	5.4884	7.4334	0.18

Table15: Bioaccumulation ranking scheme

1.	**BAF/BCF < 1000**	Not Bioaccumulative (NB)
2.	**BAF/BCF > 2000**	Bioaccumulative (B)
3.	**BAF/BCF > 5000**	Very Bioaccumulative (VB)

3.5 Derived parameters of the general linear model for bioaccumulation

Using the Stata software, a stepwise regression and 1000 iterations, Table 16 shows the following parameters, uptake rate (K_u) as 6.13kg sediment/kg fish d^{-1}, depuration Rate (K_{ee}) as 1.10d^{-1}, kinetic bioconcentration factor BCF_k of 5.98 and the steady state biota-sediment accumulation factor ($BSAF_{SS}$) of 7.74.

Table 16: Fundamental parameters from the Stata software

Parameter	Value (95%)
Uptake Rate (K_u)	6.13
Depuration Rate (K_{ee})	1.10
BCF_k	5.98
$BSAF_{ss}$	7.74

Bioaccumulation constant (BCF_k) Model
Derived equation for Bioaccumulation constant (BCF_k);
$BCF_k = 5.67 + 2.4 \times 10^{-3}$ *(time)* -2.3×10^{-3} *(conc)*

Depuration rate model
The derived equation for K_{ee} is presented below.
$K_{ee} = 2054.2 - 8.69$ *(time)* $- 21.88$ *(conc)*

Estimated 95% elimination time
Time for the substance to be eliminated at 95% from the organism = 13.6 days based on the Bioacc-model at the rate of 1.10 ug/g per day and a 15µg/g concentration.

Uptake rate model
The derived equation for uptake rate (K_u) is presented below.
$K_u = 11426.53 - 48.38(time) - 121.76(conc)$

4. Discussion

4.1 Uptake rate (k_1) of PAHs of JL-01-BLCO in test specie

The $MOSIAC_{bioacc}$ application was adapted to a Toxico-Kinetic (TK) model fitted to an accumulation and depuration data generated through the laboratory to determine BCF/BSAF, uptake rate, depuration rate, describe and predict the toxicity and the effects of PAHs of JL/01/BLCO on individuals of *Clarias gariepinus* based on experimental data. The uptake rate constant (k_1) is the numerical value defining the rate of increase in the concentration of test substance in/on test fish (or specified tissues thereof) when the fishes are exposed to that chemical (k_1 is expressed in day^{-1}). The test organisms (*Clarias gariepinus*) were exposed to PAHs of JL-01-BLCO to determine uptake rate constants for 28 days of the first phase of the bioaccumulation process. The uptake rate constant, K_1 was obtained as 6.261 kg sediment/kg fish d^{-1}. This validates *C. gariepinus* have ability to accumulate contaminants considering their bioavailability when exposed to PAHs of JL-01-BLCO. According to Lyndal *et al.* (2006), the bioavailable portion defines the amount of the total contaminant concentration that is available for uptake by the organisms. PAHs found to be hydrophobic organic compound are the main variable controlling bioavailability. Burgess *et al.* (2003) defines hydrophobicity as the tendency to be water insoluble. The increase in the hydrophobicity of PAHs has been associated with a decline in the ratio of water to sediment concentrations of PAHs as reported by Lyndal *et al.* (2006) which attributed it to the tendency of the compound to avoid water and seek a nonpolar environment. It was revealed in this study that uptake rate between Day-1 and Day-3 was most significant (62%) in test specie. However, after long-term exposure Day-7 to Day-21 least uptake rate (2%) was recorded. Bender *et al.* (1988) and Landrum (1988) demonstrated that the rates of uptake vary little over a wide range exposure of PAHs and are therefore not strongly linked to chemical hydrophobicity. The equilibrium conditions between overlying water, sediment, and the organism tissue may be immaterial because tissue deposits are expected to be a function of thermodynamics, not kinetics (Bierman, 1990).

4.2 Depuration rate (k_2) of PAHs of JL-01-BLCO in test specie

Depuration (loss) rate constant (k_2) is the numerical value defining the rate of reduction in the concentration of the test substance in the test fish (or specified tissues thereof) following the transfer of the test fish from a medium containing the test substance to a medium free of that substance (k_2 is expressed in day^{-1}). After the 28-days exposure of *Clarias gariepinus* to PAHs of JL-01-BLCO, test specie was placed in clean water environment for a period of 28 days to allow purging of the test chemical (USEPA, 2000). Therefore, the depuration rate K_2 was 1.146 d^{-1}. The elimination rate of the test chemical by *C. gariepinus* was significantly lower compared to the uptake rate. Which validates the account of several other previous researchers in this regard. *Clarias* species have shown ability to depurate/eliminate substances but tend to be slow (El- Shenawy, 2004). Babatunde *et al.* (2020) attributed this to reduction in physiological processes such as reduction in respiratory rate and movement as the organism tends to survive through avoidance mechanism and this reduces rate of elimination of contaminants as increase in ammonia and faecal excretion is associated with an increase in respiration rate (El-Shenawy, 2004). This is also in agreement with Kostopolou *et al.* (2009) who argued that if the exposure of organism is chronic, the hydrocarbon may enter more stable tissues (like lipids depot) and as long as the animal is in positive nutritional balance, it will only very slowly release the hydrocarbons. Depuration is also related to

availability of water. Okereke *et al*. (2017) posited that depuration of contaminants in shellfish (*Tympanatonus fuscastus*) was faster during rainy season than dry season, this means that for effective depuration to occur there is need for availability of source that is water which attained the safety standard. The good news is that due to low solubility of PAHs in water, they are most times adsorbed on to sediment and suspended particles hence reducing its availability to marine organisms but the organisms at the bottom is more likely to be exposed like the test organism (*Clarias gariepinus*), a sediment dweller.

4.3 Bioaccumulation factor of PAHs component of JL-01-BLCO on *Clarias Gariepinus* using bioaccumulation model

Based on the results obtained from this study, a Table of fish concentrations **(**µg/g), Time (d^{-1}), sediment concentrations (µg/g) and number of replicates, was uploaded into the $MOSIAC_{bioacc}$ application to provide the corresponding Biota Sediment Accumulation Factor (BSAF). The fish and sediment concentrations used were lipid and TOC normalized. The kinetic biota-sediment accumulation factor ($BSAF_k$) and steady state biota-sediment accumulation factor ($BSAF_{ss}$) deduced for the concentrations of Bonny light crude oil in *Clarias gariepinus* were 5.00 and 5.488. Based on the ranking scheme, the PAH component of the test substance, JL-01-BLCO is non-bioaccumulative in *C. gariepinus* since the BSAF<1000. Burckhard and Lukasewycz (2000) reported BSAFs (0.0001– 0.007) for different parent PAHs in lake trout from Lake Superior. In a study by Gewurtz *et al*. (2000) on sediments and invertebrates in Lake Erie, lipid–organic carbon normalized biota–sediment accumulation factors (BSAFs) for PAHs in mayfly larvae was found to be between (0.1–10), *Dreissena* (0.01–1), for *amphipods* (0.001–1) and crayfish (0.001–0.5). The differential accumulation among the invertebrate species was attributed to differences in biotransformation. Tuvikene (1995), James (1989) and Varanasi *et al*. (1989) reported that accumulation of PAH in fish is dependent on PAH concentration, period of exposure, and lipid content of the specie. Other factors such as water salinity, pH, hardness and temperature, size, age (young fish tend to be more susceptible) ecological need, and feeding habit play significant role too (Babatunde *et al.*, 2020). Another main criterion for bioaccumulation potential is the n-octanol/water partition coefficient (K_{ow}). The octanol/water partition coefficient has been shown to be the measure of a chemical's affinity for lipid portion of an organism's tissue (Arnot *et al*., 2004). Ubani *et al*. (2006) calculated the partition coefficient (K_{ow}) of Bonny light crude oil to be 0.743, and this tends to suggest that the crude oil will most likely not adsorb to particulate organic matter, since chemicals with K_{ow} of 2-6 are said to be lipophilic for some water body (Oliver and Charlton, 1984), it shows that Bonny light crude oil is far less lipophilic in the test microcosm aquarium (Ubani *et al*., 2006). Therefore, the speculation that chemicals with K_{ow} values ≥ 2 are lipophilic also suggests that Bonny light crude oil is not lipophilic and not liable to bioaccumulate in biota (non-bioaccumulative) and this agrees with the findings of this investigative study.

4.4 Validation of test results using developed model

In other to revalidate the results from the $\mathrm{MOSIAC_{BIOACC}}$ model as presented in this study, a model was developed to predict the uptake rate K_1, depuration rate K_2 and generate $\mathrm{BSAF_{SS}}$ based on the concentrations of PAHs in the water column, concentrations in the test specie, time of exposure and their uptake and depuration rates using the stata software alongside analysed by means of a stepwise linear regression. From the initial $\mathrm{MOSIAC_{BIOACC}}$ model, the uptake and depuration rates were reported as 6.261 kg sediment/kg fish d^{-1} and 1.146 d^{-1} respectively and $\mathrm{BSAF_{SS}}$ as 5.4884. In the case of the developed model, the determined values for uptake rate were reported as 6.13 kg sediment/kg fish d^{-1} with depuration rate of 1.10 d^{-1}, the $\mathrm{BSAF_{SS}}$ was recorded as 7.74 which shows that the PAH component of the test substance, JL-01-BLCO is non- bioaccumulative in *C. gariepinus* since the BSAF<1000, based on the ranking scheme. It is imperative to state that, the values were estimated by minimizing the error between experimental and simulated values and the results from both software showed linear similarity. To provide a general tool to evaluate bioaccumulation potentials of PAHs of BLCO on test specie, the developed model was also used to deduce a general correlation between uptake and depuration rates which largely depends on the physico-chemical properties of the test chemical. The relationships between Bioaccumulation constant (BCF_k), depuration rate ($\mathrm{K_{ee}}$) and uptake

rate (K_u) against time were estimated. It was found that, the time for the substance to be eliminated at 95% from the organism is 13.6 days based on the Bioaccumulation model at the rate of 1.10 ug/g per day and a 15µg/g concentration. The linear equations generated from the developed model are simpler ways to determine the depuration rate, uptake rate and bioaccumulation rate based on the time and concentration of the substance of exposure. However, the multiple R squared of each model were less than 0.5 (indicating a relatively lower sensitivity compared to the *Mosaicc bioacc* model).

5. Conclusion

Juveniles of *Clarias gariepinus* were exposed to sediment spiked with a sample of Bonny light crude oil coded JL-01-BLCO to evaluate the bioaccumulation potentials of the test substance (JL-01-BLCO) on *C. gariepinus* as required by test protocol. From the results obtained PAHs of JL-01-BLCO is non-bioaccumulative in *Clarias gariepinus.* That is, PAH can bioaccumulate and depurate without holding bound the contaminant in the body structure when exposed to a clean aquatic environment for a reasonable period. It is pertinent to note that the duration (time required) for bioaccumulated PAHs to be eliminated is clearly determined depending on the concentration absorbed. It should be strongly noted that depuration is not a substitute for pollution control but a process to enhance healthy living by improving safety consumption of sea food. Farmers and consumers should depurate fishes before consumption or storage as recommended by Babatunde *et al*. (2020). This is supported by the work of Irwin (1997) that human population are at risk of mutagenesis and carcinogenesis, when PAH contaminated *C. gariepinus* are consumed without adequate measures to depurate them.

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