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Toxicity Effect of Polycyclic Aromatic Hydrocarbon on the Micronucleus and Red Blood Cell of *Tilapia guineensis*

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ABSTRACT

Background and Objective: Polycyclic Aromatic Hydrocarbon (PAHs) is a recalcitrant pollutant known to have spread across the globe as a result of its anthropogenic sources. Toxic effects of Polycyclic Aromatic Hydrocarbons (PAHs) on the micronucleus and red blood cells of *Tilapia guineensis* were investigated to determine the levels and distribution of PAHs and the health risk posed to the inhabiting fauna. **Materials and Methods:** *Tilapia juvenile* weighing 25 to 29 g from the same parent stock were used for the experiment using a static-renewal bioassay protocol. The Benzo (b) fluoranthene and dichloromethane (DCM) were used as the test chemical for the bioassay. **Results:** The average number of micronucleated (MN) cells observed in the exposed *Tilapia guineensis* was 4±1.4 for DCM treatment and 1.5±0.7 for the control treatment while the one exposed to benzo (b) fluoranthene treatment had the mean concentration range from 6.5±0.5 to 7.5±10.6. Mean of binucleated (BN) cells was 3±1.4 for DCM and 7.0±5.7 to 10.5±14.8 for benzo (b) fluoranthene compared to control which had 2.0±0.0. **Conclusion:** This study indicates that the Ijegun oil spill site of the Badagry creek still shows the richness of bioactive compounds that are capable of impairing the life function of fish and its consumers.

KEYWORDS

Polycyclic Aromatic Hydrocarbon (PAHs), biomarker, genotoxicity, *Tilapia guineensis*, micronucleus

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are significant environmental pollutants that are of great concern among scientists due to their potential mutagenicity and carcinogenicity[1]. Their ability to bioaccumualte in fish organs is widely known and they are capable of long-range transport across the food chain[2]. Polycyclic aromatic hydrocarbons are a class of organic compounds produced by incomplete combustion or high-pressure processes[3]. They form when complex organic substances are exposed to high temperatures or pressures[4]. Frequently, polycyclic aromatic hydrocarbons consist of three or more fused benzene rings containing only carbon and hydrogen[5]. Differences in the configuration of rings may lead to differences in properties. Polycyclic aromatic hydrocarbons are large group of organic compounds that are included in the European Union and United States Environmental Protection Agency (USEPA) priority pollutant list due to their persistence, toxicity, mutagenic and carcinogenic properties[6,7]. A vast number of polycyclic aromatic hydrocarbons enter the environment through the atmosphere from

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incomplete combustion processes, such as processing of coal and crude oil, industrial use of coal and mineral oil products, heating of power plants and residential heating of wood and mineral oil, fires, incineration of refuse, vehicle traffic, tobacco smoking and volcanic eruptions[7].

Polycyclic aromatic hydrocarbons are classified as mutagens, carcinogens and teratogens by the United States Environmental Protection Agency[8]. Exposure of fish and other wildlife through contaminated water, soil, sediment, or food chains can result in a variety of toxic-level effects such as reduced fecundity, impaired growth and increased disease susceptibility in animals[9]. These toxic effects are classified as biomarkers that can serve as indicators of stress in the organism or the ecosystem[10]. The application of biomarkers in toxicological studies and ecological risk assessment cannot be over-emphasized as it helps to indicate the early warning signs that appear before measurable effects on the organism performance and population dynamics occurrence[11]. The prevalence of skin lesions in some fish species has been associated with exposure to polycyclic aromatic hydrocarbons from the deep water horizon oil spill in the Gulf of Mexico[12].

A lot of environmental researchers have reported oxidative stress, measured using selected biomarkers, in a fish exposed to polycyclic aromatic hydrocarbons[13] such as GSH (Reduced Glutathione level), whole body vitamin E concentrations and glutathione disulphide ratios in rainbow trout larvae exposed to the alkylated polycyclic aromatic hydrocarbons[14]. Furtherance to oxidative stress markers, other bioindicators of toxicity have been demonstrated in polycyclic aromatic hydrocarbons-exposed fish. Several developmental abnormalities associated with polycyclic aromatic hydrocarbon exposure are jaw reductions, skeletal defects, cardiac dysfunction and pericardial and yolk sac oedema[15]. Moreover, exposure of *Tilapia* fish to phenanthrene was observed to demonstrate a decrease in the hepatosomatic index as a result of adverse effects of the phenanthrene[16].

Enormous quantities of PAHs can be released into the environment by leakages or accidents in the refinery of petroleum, transport and extraction industries[17]. The environmental load remains confined when oil is spilled into the soil which leads to the collapse of entire ecosystems especially when large quantities of oil are spilled into rivers or lakes consequently due to the toxic loads of polycyclic aromatic hydrocarbons in the crude oil spillage[18]. This study is aimed at investigating the distribution and menace level of polycyclic aromatic hydrocarbons in the surface water, sediments and macrobenthic fauna of Ijegun crude oil spillage to assess the health risk posed to the inhabiting fauna as a measure of the recovery of crude oil spillage at Ijegun section of Badagry creek.

MATERIALS AND METHODS

Study area: The study was conducted around Ijegun area of Badagry creek which witnessed pipeline damage and a fire outbreak in 2008. Collection of fish, experimental setup and bioassay analysis were carried out from May to November, 2019.

Collection of fish for bioassay analysis: Juvenile *Tilapia guineensis* weighing 25 to 29 g from the same parent stock was used for the bioassay experiment.

Fish acclimatization procedure: The fishes were kept in holding tanks half-filled with water and aerated with a 220-V air pump and they were fed with 0.6 g of Coppens fish feed, twice daily. The acclimatization process lasted for a period of one week and water was changed every 3 days to prevent accumulation of toxic waste metabolites. Laboratory conditions were maintained at a temperature of 25-28°C, at a humidity of 65-75% and 10 hrs light and 14 hrs dark cycle before the bioassay in accordance with the guidelines for bioassay technique[19].

Test chemicals: Polycyclic aromatic hydrocarbon compound (Benzo (b) fluoranthene) and dichloromethane (DCM) were used for the bioassay. The chemical was purchased from Sigma-Aldrich, Germany. It was kept in a cool, dark cupboard to ensure quality preservation according to the manufacturer's instruction.

Chronic toxicity testing exercise: A total of ten acclimatized fishes were randomly caught from the stock tank using a plastic sieve and carefully transferred to the different concentration as well as the control in each bioassay tanks. The respective concentrations of the test chemical were duplicated making 5 organisms per test concentration. The test was performed to determine the sub-lethal effects. The fishes were not fed for 24 hrs before exposure. The test containers were labelled with each concentration to be introduced and were filled with 6 L of water each, while the chemical was introduced into each tank using a 1 mL/L syringe. The fishes were fed once daily and a renewal method was employed to change the water every three days, days' period.

Blood cell abnormality and micronucleus assay: Blood samples were collected from the *Tilapia* in each labelled concentrated containers using a 1mL sterile syringe. After the extraction of blood sample, a drop of the blood sample was placed on a clean glass slide. It was smeared on the slide by spreading with another slide at angle of 45°. It was air-dried and was fixed within 30 min using 70% of ethanol to ensure that the cell structure is not damaged. After the drying of the ethanol, a follow up staining was carried out using May-Grünwald stain for 10 min which was then rinsed gently in water and allowed to dry. Thereafter, a counter staining with Geimsa stain was done for 10 min and subsequently rinsed off in water and left to air dry[20].

Slide microscopy: After the staining, each slide was then ready for microscopy using a drop of oil immersion on each glass slide to be viewed. The slide was place under the microscope and its lens was adjusted to ×100 and viewed using a hand-held counter for respective shape of abnormality recording in forms like the bud shape, micronucleus, notched (bean-shaped), 8-shaped, lobbed, binucleated and polychromatophiles (PCE).

RESULTS

Red blood cell abnormalities and micronucleus induced in the blood of *Tilapia guineensis* exposed to the test chemicals are shown in Table 1.

The average number of micronucleated (MN) cells observed in the exposed *Tilapia guineensis* was 4±1.4 for DCM treatment. The control treatment had a mean concentration of 1.5±0.7 while the one exposed to benzo (b) fluoranthene treatment had the mean concentration range from 6.5±0.5 to 7.5±10.6 as compared with control treatment 1.5±0.7. The mean of binucleated (BN) cells was 3±1.4 for DCM and 7.0±5.7 to 10.5±14.8 for benzo (b) fluoranthene compared to control which had 2.0±0.0. The mean of bud shaped red blood cells was 21.5±2.1 for DCM treatment and 40.0±29.7 to 97.5±123.7 for benzo (b) fluoranthene treatments as compared with control (2±1.4). The mean of notched (N) cell was 2.5±0.7 for DCM and 3±1.4 to 4±1.4 for benzo (b) fluoranthene treatments compared with control (1.0±1.4). The red blood cells that were eight-shaped had the mean of 1.5±0.7 for DCM and 2.5±2.1 to 3±1.4 for benzo (b) fluoranthene treatments as compared with the control (0.0±0.0). The immature polychromatic erythrocytes (PCE) mean were 14±45.7 for DCM and 32.5±14.8 to 52.0±35.4 for benzo (b) fluoranthene treatments as compared with the control (3.5±0.7).

Polycyclic Aromatic Hydrocarbons (PAHs) in the environmental media and biota of Ijegun section of the Badagry creek

Sediment PAHs concentrations: The results of analysis of the sediment samples from the study area as shown in Table 2.

Table 1: Micronucelus and red blood cell abnormalities in *Tilapia guineensis* exposed to the test chemicals

		Micronucleus (MN)		Binucleated cells (BN)		Bud		Notched (N)		8-Shaped		Polychromatic erythrocytes (PCE)	
Concentration	Total (n)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Control A	1000	1	70	2	20	3	630	2	20	0	0	4	180
Control B	1000	2	40	2	20	1	560	0	0	0	0	3	400
Mean±SD	1000	1.5±0.7		2.0±0		2±1.4		1±1.4		0±0.0		3.5±0.7	
DCM A	1000	5	250	4	60	23	600	2	20	1	10	18	130
DCM B	1000	3	190	2	260	20	1690	3	20	2	40	10	120
Mean±SD		4±1.4		3±1.4		21.5±2.1		2.5±0.7		1.5±0.7		14±45.7	
1.8 A	1000	6	60	3	30	61	610	3	30	1	10	43	430
1.8 B	1000	7	70	11	110	19	190	4	40	4	20	22	220
Mean±SD		6.5±0.5		7±5.7		40±29.7		3.5±0.7		2.5±2.1		32.5±14.8	
18 A	1000	15	150	21	210	185	180	3	30	4	40	27	270
18 B	1000	0	0	0		10	100	5	0	2	0	77	770
Mean±SD		7.5±10.6		10.5±14.8		97.5±123.7		4±1.4		3±1.4		52.0±35.4	

Table 2: GC-MS analysis of PAHS level in the sediment samples from Ijegun study site

	Concentrations (mg/kg)		
PAH cmpounds	Point 1	Point 2	Point 3
Naphthalene	ND	ND	ND
Acenaphthylene	ND	ND	ND
Acenaphthene	ND	ND	ND
Fluorene	0.00480	ND	ND
Phenanthrene	0.00700	ND	0.00335
Anthracene	ND	ND	ND
Fluoranthene	0.01102	0.00281	0.00266
Pyrene	0.00366	0.00184	0.00182
Benz (a) anthracene	0.03352	0.01134	0.01554
Chrysene	0.03731	0.01267	0.01798
Benzo (b) fluoranthene	0.11278	0.03562	0.05613
Benzo (k) fluoranthene	0.09090	0.02678	0.03794
Benzo (a) pyrene	0.06139	0.02608	0.03926
Dibenz (a, h) anthracene	ND	ND	ND
Indeno (1, 2, 3-cd) pyrene	ND	ND	ND
Benzo (g, h, i) perylene	ND	ND	0.01439
Sum 16 EPA PAHs	0.36238	0.11714	0.18907
Mean±SD	0.04026±0.040128	0.01673±0.01295	0.02452±0.02125

ND: Not detected

Table 3: GC-MS analysis of PAHs level in surface water samples from Ijegun

	Concentration (mg/L)		
PAH compounds	Point 1	Point 2	Point 3
Naphthalene	ND	ND	ND
Acenaphthylene	ND	ND	ND
Acenaphthene	ND	ND	ND
Fluorene	ND	ND	ND
Phenanthrene	0.00189	0.00232	0.00159
Anthracene	ND	ND	ND
Fluoranthene	0.00219	0.00275	0.00424
Pyrene	0.00972	0.00902	0.00103
Benz (a) anthracene	0.01337	0.00781	0.00447
Chrysene	0.01730	0.00863	0.00341
Benzo (b) fluoranthene	0.05624	0.02638	0.00867
Benzo (k) fluoranthene	0.03717	0.01831	0.00764
Benzo (a) pyrene	0.03745	0.01846	0.00714
Dibenz (a, h) anthracene	ND	ND	ND
Indeno (1, 2, 3-cd) pyrene	ND	ND	ND
Benzo (g, h, i) perylene	0.01239	ND	ND
Sum 16 EPA PAHs	0.52477	0.09368	0.03819
Mean±SD	0.02086±0.01859	0.01171±0.0085	0.00477±0.0028

ND: Not detected

The impacted site for sampling sediment which is point 1 recorded the highest mean of 0.04026±0.040128 mg/kg compared to points 2 and 3 which were 100 and 200 m away from the impacted site which recorded mean concentrations of 0.01673±0.01295 and 0.02452±0.02125 mg/kg, respectively. The highest detected PAHs concentration was recorded from Benzo (b) fluoranthene in the sediment from the three sampling points which ranged from 0.05613 to 0.11278 mg/kg. Benzo (a) pyrene, benzo (k) fluoranthene, chrysene and benzo (a) anthracene were within detectable limits. However, pyrene and most of the fewer-ringed compounds were below detection level.

Surface water PAHs concentrations: The PAHs concentrations of the surface water sample from the Ijegun section of Badagry creek are as presented in Table 3.

Table 4: GC-MS Analysis of PAHs level in macrobenthic fauna sample from Ijegun

	Concentration (mg/kg)	
PAHs compounds	Periwinkle	Hermit crab
Naphthalene	ND	ND
Acenaphthylene	ND	ND
Acenaphthene	ND	ND
Fluorene	0.00216	0.00449
Phenanthrene	0.00431	0.00961
Anthracene	ND	0.00483
Fluoranthene	0.00232	0.01161
Pyrene	0.00182	0.00391
Benz (a) anthracene	0.00180	0.03219
Chrysene	0.00215	0.04014
Benzo (b) fluoranthene	0.07528	0.12129
Benzo (k) fluoranthene	0.05732	0.09783
Benzo (a) pyrene	0.05289	0.08793
Dibenz (a, h) anthracene	ND	ND
Indeno (1, 2, 3-cd) pyrene	0.01730	ND
Benzo (g, h, i) perylene	0.04472	0.10427
Sum 16 EPA PAHs	0.26387	0.51855
Mean±SD	0.02382±0.02801	0.0471±0.04623

ND: Not detected

Notably Benzo (b) fluoranthene and Benzo (k) fluoranthene had the highest concentrations. The concentration of Benzo (b) fluoranthene ranged from 0.00867 to 0.05624 mg/L at the three sampling points, with the highest value detected at point 1 (site mostly impacted) while the concentration of Benzo (k) fluoranthene ranged from 0.00764 to 0.03717 mg/L at the three sampling points with the highest value recorded at point 1. The most likely causation could be as a result of runoff from the explosion that occurred there. Also, benzo (a) pyrene, chrysene and benzo (a) anthracene were within detectable limits, while pyrene and most of the fewer ringed compounds were below detection level. The average mean concentration for sampling point 1 is 0.02086±0.01859 mg/L, for sampling point 2 is 0.01171±0.0085 mg/L and for sampling point 3 the mean record is 0.00477±0.0028 mg/L.

Polycyclic aromatic hydrocarbon concentration in the microbenthic fauna inhabiting the Ijegun section of the Badagry creek: The values from biota samples namely *Tympanotonus fuscatus* (Periwinkle) and *Clibanarius africanus* (Hermit crab) are shown in Table 4.

The less carcinogenic polyaromatic hydrocarbon of lower molecular weight (LMW-PAHs) (naphthalene, acenaphthylene and acenaphthene) were not detected in both sampled microbenthic fauna assessed, but more carcinogenic high molecular weight (HMW-PAHs). The Benzo (b) fluoranthene which was detected with highest concentration in both periwinkle 0.07528 mg/kg and hermit crab 0.12129 mg/kg. Benzo (k) fluoranthene was also recorded as the second highest in both sampled microbenthic fauna with periwinkle having 0.05732 mg/kg and hermit crab at 0.09783 mg/L. The Benzo (a) pyrene, Indeno (1, 2, 3-cd) pyrene, chrysene, were all within detectable limits in both organisms. The average mean concentration of PAHs for periwinkle was recorded at 0.02382±0.02801 and 0.04710±0.04623 mg/L for hermit crab.

DISCUSSION

This study has revealed the current status of the Ijegun section of Badagry creek in reference to the sediment, surface water and macrobenthic fauna Polycyclic Aromatic Hydrocarbons (PAHs) accumulation capacity. Physicochemical parameters of surface water at Ijegun section of Badagry creek revealed that the conditions at the sampling locations were typical of lagoons in the tropics with a surface temperature of about 30°C. The surface water pH was within the National Environmental Standards and Regulation Enforcement Agency (NESREA) limit at all three sampling points with values which ranged from 6.67 to 6.91. Dissolved oxygen, turbidity and total dissolved solids (TDS) were generally within the

stipulated NESREA limit for water bodies harbouring living organisms and it was in agreement with Doherty *et al*.[21], who study on monitoring of soil and groundwater contamination following a pipeline explosion and petroleum products spillage at Ijegun, Lagos Nigeria which also discovered that the physicochemical analysis of the groundwater samples in the year 2009 and 2010 for the pH, conductivity, TDS and the level of zinc in groundwater from contaminated and control stations were within the WHO limit for drinking water.

The results of analysis of the sediment of PAHs concentration showed that impacted site for sampling sediment which is point 1 recorded the highest mean of 0.04026±0.040128 mg/kg compared to points 2 and 3 which were 100 and 200 m away from the impacted site.

Among all the PAHs detected, the highest concentration was recorded from benzo (b) fluoranthene in the sediment from the three sampling points which ranged from 0.05613 to 0.11278 mg/kg. The benzo (a) pyrene, benzo (k) fluoranthene, chrysene and benzo (a) anthracene were within detectable limits while pyrene and most of the fewer ringed compounds were not detected. Moreover, surface water sample analysis from the Ijegun section of Badagry creek showed that Benzo (b) fluoranthene and Benzo (k) fluoranthene had the highest concentration. The concentration of Benzo (b) fluoranthene ranged from 0.00867-0.05624 mg/L at the three sampling points, with the highest value detected at point 1 (most impacted site) while the concentration of Benzo (k) fluoranthene ranged from 0.00764-0.03717 mg/L at the three sampling points with the highest value recorded at point 1 (most impacted site). Fluoranthene is the most commonly detected PAHs in water and sediments probably due to run-off from the explosion that occurred in that area, petrogenic sources, coal-tar linings of cast iron or ductile iron distribution pipes that are employed to channel wastewaters and industrial effluents[22]. According to the World Health Organization, benzo (a) pyrene concentration of 0.7 mg/L in water is harmful and this corresponds to an excess lifetime cancer risk[23].

The values of macrobenthic fauna from Ijegun section of the Badagry creek shows that the less carcinogenic polyaromatic hydrocarbons of lower molecular weight (LMW-PAHs) (naphthalene, acenaphthylene and acenaphthene) were not detected in both *Tympanotonus fuscatus* (Periwinkle) and *Clibanarius africanus* (Hermit crab), but more carcinogenic high molecular weight polyaromatic hydrocarbons (HMW-PAHs) such as Benzo (b) fluoranthene was detected with highest concentration in both periwinkle, 0.07528 mg/kg and hermit crab, 0.12129 mg/kg. The Benzo (k) fluoranthene was also recorded as the second highest in both periwinkle at 0.05732 mg/kg and hermit crab at 0.09783 mg/L. According to Abrajano *et al*.[24], the ratio of high molecular weight PAHs (HMW-PAHs) to low molecular weight PAHs (LMW-PAHs) has been used to characterize the origin of PAHs in the environment to know whether it's from petrogenic source or pyrogenic source. Petrogenic sources are derived from petroleum, such as natural oil seepage and oil spills while pyrogenic sources are derived from the combustion of petroleum, automobile tire and wood and vehicle emission[25]. Nevertheless, PAHs may be transported from their points of release to the coastal environment via surface runoff and atmospheric deposition. The sources of PAHs in the studied area were both petrogenic and pyrogenic sources. The more carcinogenic HMW-PAH, Benzo (a) anthracene, Benzo (b) fluoranthene and Benzo (k) fluoranthene were reported in relatively higher concentration from this study and it was in agreement with the work of Benson *et al*.[26] whose studies focused on the occurrence and distribution of polycyclic aromatic hydrocarbons in surface microlayer and subsurface seawater of Lagos Lagoon.

Furthermore, the 28 days exposure of juvenile *Tilapia guineensis* to sub-lethal concentrations of Benzo (b) fluoranthene was observed to increase the number of micronucleated cells (MN) which showed nuclear abnormalities such as swollen cells, hemolyzed cells, notched nucleus, swelling of binucleated cell, micronucleus, lobed and blebbed polychromatic erythrocyte which may be due to a response of the fish

against the test chemical compound and it is in agreement with the study of Ivanova *et al.*[27], who reported that the swelling of red blood cell is as a result of hypoxic conditions, osmotic or macrocytic anemia in fishes exposed to pollution. Nuclear abnormalities are considered to be indicators of genotoxic damage[28]. In a related study, induction of the micronucleus and nuclear abnormalities in the peripheral erythrocytes of *Clarias gariepinus* treated with process water were observed[29]. The result of micronucleus assay from this study equally showed higher bi-nucleated cells than micronucleated cells and it was in contrast to the study of Bekibele *et al.*[30], who evaluated the toxic effects of crankcase oil exposure and meta-analysis of fish. The species showed varying degrees of micronuclei and bi-nucleated frequencies in their peripheral erythrocytes. This study therefore recommends the need to improve dredging and clean-up activities in areas of pipeline bursts or explosions in order to minimize pollution and contamination of water body and its inhabitants. Holistic regulations should also be enforced by the necessary agencies with overall pollution reduction efforts and not for mere fund generation, which will ensure sustainable protection of aquatic environment.

CONCLUSION

The findings from this study indicate that the Ijegun oil spill site of Badagry creek still shows the richness of bioactive compounds that are capable of impairing the life function of fishes and their consumers. However, the effects observed in the wild may not be as dramatic as those in this study owing to dilution factors, which remain to be proven. Therefore, this study calls for a wider ecological investigation in which the fishes of the lagoon can be assessed for a number of effects of pollutants including those examined in this study so as to allow for a proper understanding of the current situation.

SIGNIFICANCE STATEMENT

This study discovered that there is still an abundance of bioactive compounds at Ijegun oil spill site of the Badagry creek capable of impairing the life function of fish and its consumers. Having prior knowledge and understanding of the presence of these environmental pollutants could be beneficial to researchers in terms of exploring and curtailing their potential mutagenicity and carcinogenicity. Therefore, the toxic effect of PAHs on exposed fish could serve as essential biomarkers for the detection of petroleum effluent pollution from the crude oil spill and also be used in biomonitoring prog by the government agencies saddled with the responsibility of regulating crude oil explorations and pollution control. Thus creating a new frontier of assessing the environmental risk of those pollutants.

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