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Investigation of Toxicity in Black Jaw Tilapia (*Sarotherodon melanotheron*) Exposed to Crude Oil

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The toxicity effects of crude oil were investigated in the laboratory. Triplicates of (T_1) 0.25mg/l, (T_2) 1.0mg/l, (T_3) 2.25mg/l, (T_4) 5.0mg/l and (T_5) 7.5mg/l concentration of crude oil exposed to *Sarotherodon melanotheron* species. The acute toxicity test of crude oil when tested against *Sarotherodon melanotheron* revealed that the derived toxicity index LC_{50} was 0.925mg/l. On computing Toxicity Factor (TF), using 96 hours. LC_{50}, crude oil was found to be very toxic to the *Sarotherodon melanotheron* juvenile. The mean frequencies of micronucleus in *S. melanotheron* exposed to different concentration of crude oil ranged from 3.01±0.50 – 27.48±2.71. The lowest value was 3.01±0.50 in T_0 (control) while the highest value of 27.48 ± 2.71 was recorded in fishes exposed to (T_5) 7.5mg/l test solutions. The results obtained from micronucleus test showed that T_5 had the highest number of micro-nucleated cells followed by T_4 while T_1, T_2, T_3, and T_0 significantly increased with the concentration across the test chemical. *Sarotherodon melanotheron* showed various degrees of sensitivity in monitoring genetic damage especially in the normal nucleus (NN). The chromosomal aberrations indicate formation of vacuolated nucleus (VC), micronucleus (MN) and bi-nucleated cells (BN) showed marked increase in occurrence in the following concentrations of occurrences; T_1, T_2, T_3, T_4 and T_5, respectively. Test solution of concentration T_5 (7.5mg/l) was observed to possess fish with highest level of micronucleus frequencies followed by T_4 (5.0mg/L). There were significant differences in increasing T_5 having the highest number of micro-nucleated cells (MN) with a trend in increasing bi-nucleus cells (BN), polymorphic nucleus (PM), kidney shape nucleus (KN) and bleb nucleus (BLN) cells, respectively, as the concentration of the test chemical increased. The results also showed that there was a significant difference in the effects of the T_5 and other test concentrations (T_1, T_2, T_3, T_4 and T_0). The response of *Sarotherodon melanotheron* established that is a better model for bioassay test used as a pollution bio indicator. Pollutants even in a very low concentration if present for a long duration may affect the nucleus. Hence, the use of any kind of substances such as crude oil products and synthetic chemicals in aquaculture field should be carefully monitored and used under proper guidance.

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INTRODUCTION

Aquatic environments are loaded with several types of inorganic and organic pollutants. Globally, concerns on ecological safety and conservation of aquatic organisms due to residual oil have increased and remain a serious challenge in the environment [1]. Hydrocarbons, nitrogen, sulphur and metals are naturally constituent of crude oil [2]. Crude oil consists of volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs). All crude oil contains valatile VOCs, into the air causing harm to organism in water and giving crude oil an unpleasant odour while PAHs can accumulate in the environment for long period of time and may cause harm to organisms. Oil spills have caused serious threat to the environment of the oil producing areas, which if not effectively monitored can lead to the destruction of ecosystems [3]. Oil spill is poisonous and harmful to aquatic organisms. For example, in Nigeria, the Niger Delta is among the most important aquatic environment globally [4]. The main sources of oil spill in the Niger Delta are; both accidental and deliberate, from oil tankers on the high sea and the disposal of used oil into the drains by the road side mechanics, vandalization of the oil pipelines by the local inhabitants; ageing of the pipelines; oil blow outs from the flow stations [5]. Crude oil can be lethal in acute or chronic levels and can lead to high mortality of the aquatic biotic components due to toxic chemicals in the crude oil and its water soluble fraction [WSF] [6]. According to Ayoola and Taoreed [7], these can cause irregular movement, increased biological activities, imbalance of equilibrium, and eventually death in fish. Crude oil toxicants enter the body system of aquatic animals (Fishes) through the gills, digestive tract and general body surface causing significant damage to the internal organs and tissues

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during oil spill or leakages [8]. Exposure of cod embryos to crude oil dispersions caused acute and delayed toxicity, such as manifestation of physical morphological deformations in hatched larvae, spinal deformations as well as alterations in craniofacial and jaw development and finally, death [9]. *Sarotherodon melanotheron* belong to the family *Cichlidae* which serves as an important source of food in tropical and subtropical Africa. *S. melanotheron* is valuable for investigating toxic substances in the aquatic ecosystem. It was reported that the chronic carcinogenetic bioassays with small fish species are feasible and scientifically valid [4]. Cell based toxicity models can provide a sensitive and reliable toxicity assessment, while avoiding complications arising through the use of convectional animal –based toxicological screening studies [10]. Fish has been used and proved as a major bio indicator for environmental contamination, providing evidence for transmission of pollutants in aquatic environment [11]. This study was to determine the LC_{50} at 96 hours of exposure and to evaluate the genotocixity effects of crude oil on the genetic materials of *S. melanotheron.*

MATERIALS AND METHODS

Experimental setup

The study was carried out at Department of Marine Sciences Aquatic toxicological laboratory at the Biological Garden of University of Lagos, Akoka, located between Longitude 30 23′E and 30 53′E, and Latitude 6^0 26′N and 6^0 37′N in Lagos state, Nigeria. Post juvenile Black-chin Tilapia (*Sarotherodon melanotheron*) was purchased from Agboola Fish farm located at Ayobo, Lagos and transported to the research facility in aerated plastic container. The fishes were acclimatized to laboratory conditions for 14 days in large tanks. The fishes were fed daily with Bluecrown feed during acclimatization. The crude oil was purchased from Fidton Petroleum Limited located at, Amuwo Odofin Lagos.

Bioassay procedure

Acute toxicity testing *Sarotherodon melanotheron* was exposed to different concentrations of crude oil which was observed to be toxic and mortality was recorded in the treatment with the highest chemical concentrations for the range finding test. The definitive test was carried out using 7L of dechlorinated water and the concentrations were recorded. The 96 h LC_{50} value was determined by exposing *Sarotherodon melanotheron* to crude oil, acute toxicity bioassays were conducted in plastic tanks of 50 x 30 x 30m in static renewal laboratory system with the test solution changed every other day to maintain a constant crude oil concentration. Ten fishes were randomly exposed to each test concentrations obtained by exposure to crude oil and a control. The experiment was set in triplicates to obtain the 24, 48, 72, and 96 h LC_{50} value of *Sarotherodon melanotheron* exposed to crude oil. During the acute toxicity testing, the mortality of *Sarotherodon melanotheron* was recorded after 24, 48, 72, and 96 hours under each test concentrations.

Sublethal testing This bioassay was conducted for 28 days and the static renewal method was adopted. The solution was replaced with freshly prepared media concentration for every 24 hours. Juveniles *Sarotherodon melanotheron* were exposed to sub-lethal concentration of crude oil: 0.00, 0.25, 1.00, 2.50, 5.00 and 7.50mg/l, respectively. Thirty fishes per treatment were set in triplicates and control. A total of 180 test fishes were exposed per sub-lethal concentrations including control.

Each test medium was changed into a fresh solution of exactly the same concentration of the crude oil and untreated control respectively every 48 hours, same exposed test animals were transferred into freshly prepared test mediums.

Genotoxicity analysis of *Sarotherodon melanotheron* exposed to sub-lethal concentration of crude oil

Fishes are randomly selected from all the treatment and blood samples were collected and smeared on microscope slides, they were then fixed, stained with Giemsa (sigma) solution, they were then rinsed with ethanol and then left to air dry over night before examining the slides with Microscope using oil-immersion (x1500). For the scoring of micronuclei, the methods were adopted from literature [12].

Statistical analysis

All statistical analyses were conducted using Graph pad prism 5.0 computer programs. Data were presented as mean ± standard error (SE). One-way analysis of variance (ANOVA) was used to determine the differences among various groups, while Duncan multiple post hoc tests was used to compare the level of significance ($p< 0.05$) of each treated group with the negative control. LC_{50} means the concentration that kills 50% of the test population while LC_{95} means the concentration that kills 95% of the test population. TF denotes Toxicity factor for relative potency measurement.

RESULTS

The analysis of the concentration mortality data of crude oil when tested against *S. melanotheron* revealed that the derived toxicity indices (LC_{50}) is 0.925mg/l on the basis of computed Toxicity Factor (TF), using 96 hours LC_{50}, crude oil was found to be very toxic to the *S. melanotheron* juvenile and this was presented in Table 1 and Figure 1.

TABLE 1. Percentage mortality of *Sarotherodon melanotheron* juvenile exposed to crude oil

Concentration (ml)	96 hours LOG Concentration	No. of Fishes	No. Responding	Mortality, %	Probit Values
0	0	30	0	0	0
5	0.699	30	2	6.67	3.52
7.5	0.8751	30	3	10	3.72
10	1	30	10	33.33	4.56
12.5	1.097	30	19	63.33	5.33
15	1.1761	30	30	100	8.09

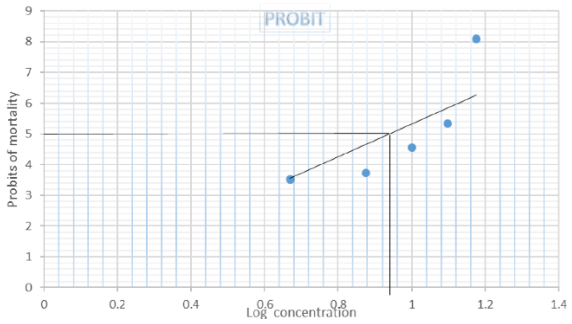

Figure 1. Probit of mortality against log concentration of crude oil *Sarotherodon melanotheron* at 96h LC_{50}

The analysis of the concentration mortality data of crude oil when tested against *Sarotherodon melanotheron* revealed that the derived toxicity indices (LC_{50}) is 0.925mg/l on the basis of computed Toxicity Factor (TF), using 96 hours LC_{50}, crude oil was found to be very toxic to the *Sarotherodon melanotheron* juvenile. The Mean frequencies of micronucleus in *Sarotherodon melanotheron* exposed to different concentration of crude oil ranged from (3.01 – 27.48) summarized in Table 2. The lowest value was (3.01) and recorded in organisms exposed to T_0 (control) experiment and highest value was (27.48) and recorded in fishes exposed to T_5 (7.5 ml/l) test solutions. The results obtained for micronucleus showed that T_5 had the highest number of micro-nucleated cells followed by T_4 while T_1, T_2, T_3, and T_0 significantly increases in micro-nucleated cells as the level of the concentration increases across the test chemical. *Sarotherodon melanotheron* specie showed various degrees of sensitivity in monitoring genetic damage especially in the normal nucleus (NN). This is indicated by variations in averages of the micro-nucleated cells among species at various test concentrations. The chromosomal aberrations represented by the formation of normal nucleus (NN), vacuolated nucleus (VC), micronucleus (MN) and bi-nucleated cells (BN) showed marked increase in the following concentrations of occurrences; T_1, T_2, T_3, T_4 and T_5, respectively. Test solution of concentration T_5 (7.5 mg/l) was observed to possess fish with highest level of micronucleus frequencies followed by T_4 (5.0mg/l). There were significant differences in increasing T_5 having the highest number of micronucleated cells (MN) with a trend in increasing bi-nucleus cells (BN), polymorphic nucleus (PM), kidney shape nucleus (KN) and bleb nucleus (BLN) cells, respectively, as the concentration of the test chemical increases. The results

Table 2. Mean frequencies of different nucleated cells in erythrocytes of *Sarotherodon melanotheron* exposed to crude oil

C	T1(0.25ml)	T2(1.00ml)	T3(2.50ml)	T4(5.00ml)	T5(7.5ml)
NN	54.75 ± 6.53^{b}	49.17 ± 6.23^{ab}	44.19 ± 6.10^{ab}	35.34 ± 4.08^{c}	17.65 ± 1.63^{d}
PN	1.00 ± 0.00^{a}	2.00 ± 0.00^{a}	2.71 ± 0.31^{a}	3.33 ± 0.42^{a}	3.91 ± 0.43^{a}
SN	1.50 ± 0.24^{a}	2.33 ± 0.33^{a}	3.75 ± 0.72^{a}	1.80 ± 0.18^{a}	3.25 ± 0.42^{a}
KN	3.33 ± 0.42^{a}	4.75 ± 0.95^{a}	5.18 ± 1.03^{a}	6.78 ± 0.95^{a}	8.68 ± 0.65^{a}
BLN	3.75 ± 0.72^{a}	5.18 ± 1.03^{a}	7.13 ± 1.05^{a}	7.50 ± 0.82^{a}	9.00 ± 1.12^{a}
BN	4.67 ± 0.64^{a}	6.62 ± 1.14^{a}	11.91 ± 0.72^{b}	18.02 ± 1.05^{b}	21.72 ± 1.18^{ab}
MN	5.77 ± 0.71^{b}	7.00 ± 0.85^{b}	16.91 ± 1.95^{ab}	22.64 ± 1.57^{bc}	27.48 ± 2.71^{bc}
VC	15.50 ± 2.96^{a}	10.76 ± 1.64^{ab}	9.67 ± 0.98^{a}	5.57 ± 0.73^{a}	4.72 ± 1.61^{ab}

*Mean ± SE values (superscript in each row of the same alphabet are not significantly different ($p<0.05$). NN= Normal nucleus, PN= Polymorphic nucleus, SN= Segmented nucleus, KN= Kidney shaped nucleus, BLN= Bleb nucleus, BN= Binucleus cells, MN= Micronucleus, VC= Vacuolated nucleus.

also showed that there was a significant difference between the T_5 and other test concentrations (T_1, T_2, T_3, T_4 and T_0) for polymorphic nucleated cells (PM), segmented nucleus (SM), kidney shape nucleated cells (KN) and bleb nucleated cells (BLN), respectively. The vacuolated nucleus cells (VC) reveal that there is a significant difference ($p<0.05$) among the test chemicals as it decreases along with increasing the concentrations. The micronucleus and nuclear abnormalities are presented in plates 1 – 8 (In Figure 2).

DISCUSSION

The acute toxicity result showed that mortality rate increased with an increase in the concentration of crude oil. The analysis of the concentration mortality data of crude oil when tested against *Sarotherodon melanotheron* further confirms the high toxicity of crude oil since the derived toxicity indices (LC_{50}) is 0.925mg/l on the basis of computed Toxicity Factor (TF), using 96 hours LC_{50}, crude oil was found to be very toxic to the *Sarotherodon melanotheron* juvenile.

The result of this study showed that crude oil has considerable effects on the blood, gills, muscle and kidney of the juvenile *Sarotherodon melanotheron* and is in concordance with literature [13], who worked on the impact of refined petroleum spills on water quality macro-invertebrates and microbial communities of a test organism. *Sarotherodon melanotheron* exposed for 28 days to various sub-lethal concentrations of water soluble fractions of crude oil grew significantly less than unexposed fish. In a similar study, pink salmon exposed to sub-lethal concentrations of water soluble fractions of crude oil for 40 days were significantly smaller than the control [14, 15]. *Sarotherodon melanotheron* showed a considerable increase in frequency of micronucleus in the T_5 (7.5mg/l) as well as in the test solution exposed to sub-lethal concentration of crude oil.

The alterations in the structure of DNA as a result of exposure to pollutants may either be irreversible, giving rise to mutations and cell death, or be repaired by various DNA repair enzymes without producing toxic effects. Micronuclei were absent in control and present in the graded concentrations of exposed fishes and erythrocytic nuclear abnormalities in the peripheral blood of *Sarotherodon melanotheron*.

The micronucleus test and the erythrocytic nuclear abnormalities are considered as powerful tools for monitoring the environment for the presence of cytotoxic agents. The frequency of micronuclei has been proved very reliable test for studying cytotoxicity in vivo and in vitro hence making it possible to compare the results obtained in the laboratory with that in the natural ecosystem. In the present study, there were no micronuclei abnormalites seen in the control but in the test concentration of exposed fishes, crude oil causes some nuclear abnormalities in the nucleus of erythrocytes and increases the form of micronucleus as the test concentration increases from T_1 to T_5. The actual and exact mechanism for the formation of erythrocytic nuclear abnormalities is not fully understood as reported by Çavaş and Ergene-Gözükara [16].

During the sub-lethal exposure period, as the concentration of exposure increases with an increase in the frequency of micronuclei was seen in 28 days exposure and was highest on T_5 (7.5ml) test concentration, other nuclear abnormalities that showed

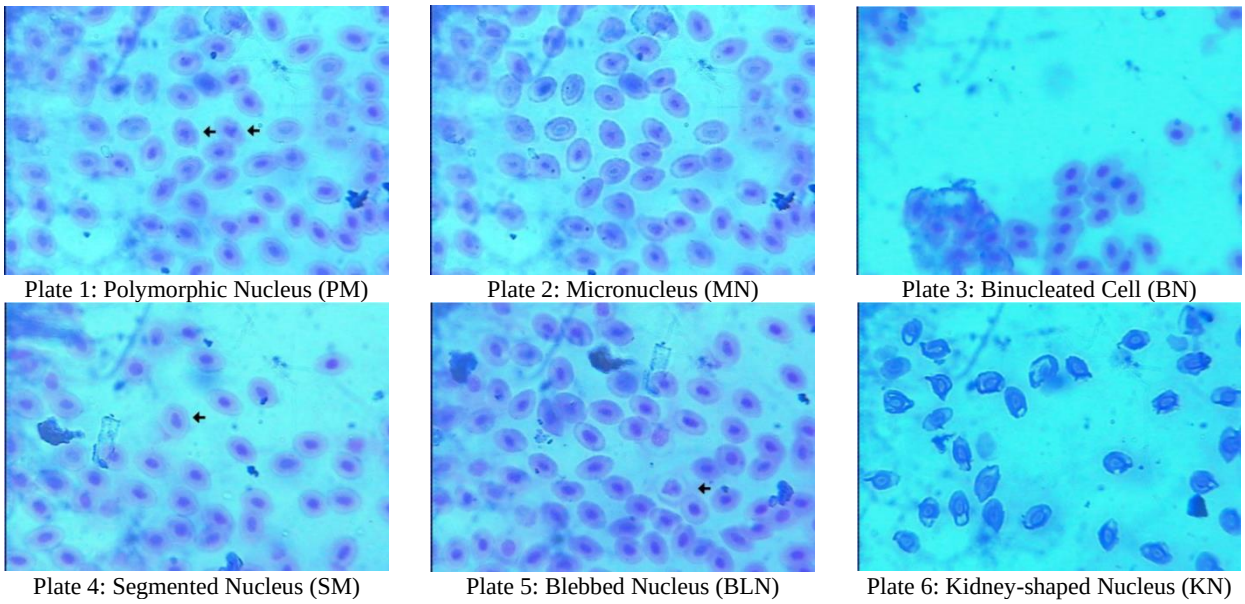

Plate 1: Polymorphic Nucleus (PM) | Plate 2: Micronucleus (MN) | Plate 3: Binucleated Cell (BN)

Plate 4: Segmented Nucleus (SM) | Plate 5: Blebbed Nucleus (BLN) | Plate 6: Kidney-shaped Nucleus (KN)

Figure 2. Plates 1-6: Micronucleated (MN) and nuclear abnormalities observed in crude oil - treated *Sarotherodon melanotheron*

increase in their frequency with increase in concentration were polymorphic nucleus (PN), kidney-shape nucleus (KN), bleb nucleus (BLN) and bi-nucleus (BN). This was similar with the reported data in literature [17] who worked on fish species exposed to petroleum and other distillate products and to other toxicant compounds. However, nuclear abnormalities were also observed in the erythrocytes obtained from different fish species on exposure to different kinds of clastogenic and aneugenic compounds and similar to the work of [18–20].

CONCLUSION

Crude oil showed a considerable increase in frequencies of micronucleus abnormalities and some structural abnormalities in the red blood cells of Sarotherodon. melanotheron during sublethal exposure to crude oil at the lowest concentration of (0.25mg/l). Pollutants even in a very low concentration if present for a long duration of time may affect the nucleus and cause genetic material damage. The responses of Sarotherodon melanotheron to the sublethal treatment effectively indicate this fish as bio monitoring model to assess the genotoxicity of contaminants and environmental health.

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چکیده

اثرات سمیت نفت خام در آزمایشگاه بررسی شد. نمونه‌های 0.25mg / l (T_1)، 1.0mg / l (T_2)، 2.25mg / l (T_3)، 5.0mg / l (T_4) و 7.5mg / l (T_5) غلظت نفت خام در معرض گونه‌های *Sarotherodon melanotheron* قرار گرفت. آزمایش سمیت حاد نفت خام هنگام آزمایش در برابر *Sarotherodon melanotheron* نشان داد که شاخص سمیت مشتق شده LC_{50} 0.925mg / l بود. در محاسبه فاکتور سمیت (TF)، با استفاده از *Sarotherodon melanotheron* طی ۹۶ ساعت مشخص شد که روغن خام LC_{50} برای نوجوانان بسیار سمی است. میانگین فرکانس‌های میکرو هسته در *Sarotherodon melanotheron* در معرض غلظت‌های مختلف نفت خام از ۲۷/۴۸ ±۲/۷۱ – ۳/۰۱ ±۰/۵۰ بود. کمترین مقدار ۳/۰۱ ±۰/۵۰ در T_0 (شاهد) بود در حالیکه بالاترین مقدار ۲۷/۴۸ ±۲/۷۱ در ماهیان در معرض محلول‌های آزمایش ۷/۵ mg/ l (T_5) ثبت شد. نتایج به دست آمده از آزمایش میکرو هسته نشان داد که نمونه T_5 دارای بیشترین تعداد سلول‌های میکرو هسته‌ای و به دنبال آن نمونه T_4 است در حالی که نمونه‌های T_1، T_2، T_3 و T_0 با غلظت در سراسر ماده شیمیایی آزمایش به طور قابل توجهی افزایش می‌یابد. *Sarotherodon melanotheron* در نظارت بر آسیب ژنتیکی به ویژه در هسته طبیعی (NN) حساسیت درجات مختلفی را نشان داد. انحرافات کروموزومی نشان می‌دهد تشکیل هسته واکوئله (VC)، ریز هسته (MN) و سلول‌های دو هسته‌ای (BN) افزایش قابل توجهی از وقوع را در غلظت‌های زیر از وقوع نشان داد، به ترتیب T_1، T_2، T_3، T_4 و T_5. محلول آزمایش غلظت 7.5mg / l (T_5) برای داشتن ماهی با بالاترین سطح فرکانس‌های میکرو هسته‌ای و به دنبال آن 5.0mg / L (T_4) مشاهده شد. تفاوت معنی‌داری در افزایش T_5 با داشتن بیشترین تعداد سلول‌های میکرو هسته‌ای (MN) با روند افزایش سلول‌های دو هسته‌ای (BN)، هسته چند شکلی (PM)، هسته شکل کلیه (KN) و هسته مخلوط (BLN) وجود داشت. همچنین نتایج نشان داد که اختلاف معنی‌داری در اثرات T_5 و سایر غلظت‌های آزمایش (T_1، T_2، T_3، T_4 و T_0) وجود دارد. پاسخ *Sarotherodon melanotheron* نشان داد که مدل بهتری برای آزمایش سنجش به عنوان شاخص زیست آلودگی استفاده می‌شود. آلاینده‌ها حتی در غلظت بسیار کم اگر در مدت طولانی وجود داشته باشند، ممکن است هسته را تحت تأثیر قرار دهند. از این رو، استفاده از هر نوع موادی مانند فرآورده‌های نفتی خام و مواد شیمیایی مصنوعی در زمینه پرورش آبزیان باید به دقت کنترل و مورد استفاده قرار گیرد.