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Genotoxic Evaluation and Toxicity of Spent Engine Oil on *Clarias gariepinus*

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ABSTRACT

In most of developing nations, pollution of water resources has become a serious problem. Apparently, human and ecological disorder experienced in industrial settlements as a result of improper disposal of chemicals such as engine oil calls for careful surveillance on the state of the environment. The acute toxicity concentrations of 100, 200, 300, 400 and 0 mL L^{-1} were used to determine the 96 h Lethal Concentration (LC_{50}) value of spent engine oil which was found to be 2.75 (562 mL^{-1}). Definitive test was also carried out every 24 h over a four days period (96 h). Cytogenetic evaluation using micronucleus assay was carried out on *Clarias gariepinus* juveniles in the laboratory after exposure to sub lethal concentrations of spent engine oil for 14 days. The One way ANOVA was used to analyze the significant difference ($p<0.05$) in the analysis of variance for micronucleus and bi-nucleated cells. Micronucleus assay showed more bi-nucleated cells than micro nucleated cells in *Clarias gariepinus* juveniles exposed to sub lethal concentrations of spent engine oil. The species showed varying degrees of micronuclei and bi-nucleated frequencies in their peripheral erythrocytes. Cytological examinations showed bi-nucleated cells and micronucleus formation in erythrocytes of the fish in the test solution. However, the significant difference was not wholly dependent on the period of exposure and the concentration of spent engine oil used. The results show that spent oil commonly discharged in the environment is capable of causing genetic damage to *Clarias gariepinus* at high concentrations of the assay; this can be employed for the evaluation and the assessment of water pollution and aquatic mutagens. Spent engine oil is toxic to fish and causes cytogenetic changes in cells of fish. Fish are susceptible to spent engine oil; therefore the release of spent engine oil into the aquatic environment should be discouraged.

Key words: Genotoxic, toxicity, spent engine oil, *Clarias gariepinus*

INTRODUCTION

Human activities in agriculture, industry, power generation, urbanization and transportation result in the emission of several wastes products in solid, liquid or gaseous forms into the aquatic environment (Ayoola, 2008). In many developing countries such as Nigeria, the activities of informal mechanical workshops, usually results in uncontrolled discharge of spent engine oil into drainage channels and canals and this eventually end up in the aquatic bodies. The indiscriminate discharge of spent engine oil into the environment and its negative effects on the environment demands the development of various control strategies. Mechanical workshops now use dispersants, emulsifiers, degreasers or detergents in washing off spent engine oil thus ensuring easy dispersal. Dispersants are imported into the Nigeria from Europe, North America, Asia and other temperate countries. In spite of legislation limiting the disposal of toxic chemicals, pollution of aquatic environments still occurs (Fleeger *et al.*, 2003). Aquatic species are not equally susceptible to toxic substances and much information on the toxicities of spent engine was based on species not native to Nigeria and in environmental conditions different from those of Nigeria. The Department of Petroleum Resources (DPR), the apex body in Nigeria regulating activities of the petroleum industries has since 1980, demanded 96 h LC_{50} toxicity tests of drilling mud systems, base oil, oil-based muds, chemical lubricant and degreaser on local species under Nigerian conditions to determine the safety of their use before these chemicals are approved to be used in the petroleum industry in Nigeria (Odiete, 2003).

Spent engine oil which is chemically referred to as used mineral-based crankcase oil is simply described as the brown to black, oily liquid removed from the engine of a motor vehicle when the oil is changed. It is consists of branched alkanes; cycloalkanes; Polyaromatic Hydrocarbons (PAHS); linear alkanes; decomposition products; additives and contaminants of worn engine parts which include heavy metals such as chromium, iron, lead, nickel, silicon, tin, aluminum, copper, magnesium. Spent engine is a common and toxic environmental contaminant that is not naturally found in the environment. However, disposal of spent engine oil into gutters, water drains, open vacant plots and farms is commonly practice in Nigeria, especially by motor mechanics that change oil from motor vehicles and generators (Fleeger *et al.*, 2003).

Many environmental contaminants exert their effects via genotoxic and metabolically toxic mechanisms simultaneously causing carcinogenesis, embryo toxicity and implicit a long term alteration in organisms by active through several generations (Ali *et al.*, 2008). Environmental contaminants has developed different methods for double and single strand breaks of DNA, DNA-adducts, micronuclei formation and chromosome aberrations. One of the most popular and promising is the micronucleus test (MN). It is a marker of cytogenetic damage usually caused by clastogenic or aneugenic compounds. The assessment of cytogenetic damage has been presented assay in identification of pollution hazards in marine environment.

In fish, the kidney is responsible for erythropoiesis as well as filtration. Upon fish exposure to toxins, defective erythrocytes undergo passage from the kidney into the peripheral blood, from where they are removed by the hemocatheresis organs (Palhares and Grisolia, 2002). Chromosomal aberration studies with preponderant native fish species represent an important effort in delineating the extent of a particular chromosome damage and change such as micronuclei formations and likely agents inducing the visible aberration in the fish genome.

According to Odiete (2003), test organism to be used for toxicity test must be ecological and economically important, occupy trophic positions leading to humans or other important species, have adequate background biology, be widely distributed, be genetically stable, have its early stages (larvae, fry, juveniles) available throughout the year and be sensitive. The African catfish *Clarias gariepinus* is an ecologically important and commercially valued fish in Nigeria (Ayoola, 2008). These mudfish are frequently and widely cultured in ponds and they also occur freely in Nigerian natural freshwaters. *Clarias gariepinus* has been chosen because it is sensitive being in its early life stage when compared to the adult (Odiete, 2003). This study intend to investigate the acute toxicity of spent engine oil using *Clarias gariepinus* and evaluate the genotoxic effects of spent engine oil using micronucleus assay in *Clarias gariepinus*.

MATERIALS AND METHODS

Clarias gariepinus of 8.0±0.2 cm in length and with an average weight of 7.8±0.2 cm were bought from a private fish farm in Jakande estate area of Lagos state. They were placed in a plastic container which was properly aerated and transported to the marine sciences laboratory at the University of Lagos.

The fish were kept in a glass tank (50×30×30 cm) for two weeks in properly aerated water. The stocking water was changed every day and the juveniles were fed twice daily with commercially prepared feed obtained from the fish farm until, feeding was stopped a day before the bioassay test.

Test compound: spent engine oil: Spent engine oil was collected in gallons from different mechanic workshops and fuel stations located in the Osodi-Isolo metropolis for the bioassays on *Clarias gariepinus* juveniles. The spent oil collected from different mechanic workshops were mixed uniformly by adding equal volumes of oil from each source into the same container and this served as the test compound.

Bioassay procedure: The preliminary tests were carried out at first to determine suitable range of concentration on the *Clarias gariepinus* juveniles for 96 h that four days. The concentration ranges for the range finding test were 100, 200, 400, 60, 70 and 900 mL^{-1}. these concentrations were carefully measured out with a measuring cylinder to make out the correct measurements in triplicate of six plastic containers. While clear undiluted water served as control. In each of these bowls, 10 juveniles of 8.0±0.2 in length with mean weight of 7.8±0.2 g were introduced.

Assessment of quantal response (mortality): Mortality assessment was carried out every 24 h over a 96 h range finding experimental period. Fish was assumed to be dead when there was no body or operculum movement, even when prodded with a glass rod. The results gotten from the range findings test are used in calculating the definitive test as described by Ayoola (2008). Definitive test was also carried out every 24 h over a four day period (96 h). Ten active animals were introduced into the test medium containing spent engine oil. Each treatment was set in triplicates, giving a total of 30 fishes per test medium, including untreated media (control). The test animals were exposed to the following concentrations of the spent lubricant oil: Spent lubricant oil: 400, 500, 600, 700, 800 and 0 mL L^{-1} (control) v/v. These values were generated from the range finding experiment.

Cytogenetic analysis of *Clarias gariepinus* exposed to sublethal concentration of spent lubricant oil: In this analysis, *Clarias gariepinus* juveniles were exposed to sub lethal concentration of spent lubricant oil as follows: Spent lubricant oil: 100, 200, 300, 400 and 0 mL L^{-1} (control). These values were generated from the definitive test experiment.

Each treatment was set in triplicates, giving a total of 30 fishes per treatment including untreated media (control). A total of 150 test animals were exposed per sub lethal concentrations including control. The sub-lethal toxicity tests carried out for 14 days was to investigate the rate at which *Clarias gariepinus* accumulates spent engine oil. The semi-static renewal bioassays procedure was adopted. To avoid drastic changes in concentration of test media via evaporation and reduction in dissolved oxygen levels, the test media were removed from their respective containers once every twenty-four (24 h) and replaced into freshly prepared test media over a fourteen day (14 day) period of sub lethal experimentation.

At determined time intervals,(day 7 and 14), five (5) live *Clarias gariepinus* were randomly selected from each concentration of a triplicate including control, blood samples were collected from the selected fish 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 following criteria were adopted from Campana *et al.* (2003); the diameter of the micronucleus marginally overlap with main nucleus as long as there is clears identification of the nuclear boundary and MN should have similar staining as the main nucleus.

Micronuclei analysis: Blood samples were obtained by caudal vein puncture using a heparinized syringe and directly smeared on microscopic slides. The microscope slides were air dried for 24 h fixed in methanol for 20 min and left to air dry for twenty-four hours and stained in 10% Giemsa (sigma) solution for 20 min and left to air dry for twenty four h The stained slides were analyzed under a light microscope at a magnification of 1000x. Micronucleus was identified according to the following criteria:

Spherical or ovoid-shaped extra nuclear bodies in the cytoplasm, diameter of 1/3-1/20 of the main nucleus on-refractory bodies, color texture and optical features resembling those of the nucleus and the bodies are completely separated from the main nucleus.

Physicochemical parameters of the test media: The pH, temperature and dissolved oxygen content of the control and test media in the experimental set-up were determined. Temperature measurement was determined in situ by means of a simple mercury-in-glass thermometer. A highly sensitive pH meter (Philips pH meter model 9405) with glass electrode was used for the determination of pH sample. The Dissolved Oxygen (DO) was measured using appropriate digital instruments (Horiber U-10).

Acute toxicity test: The probit analysis was generated through a computer generated programme designed and implemented by Ge-le Pattaurriel Imperial College, London run by an IBM computer. The values against log-dose value for LC_5, LC_{50} and LC_{95} were obtained and tabulated graphs of probit values against log dose values were obtained and tabulated. Graphs of probit values against log values were plotted using the line of best fit for a straight line curve. The following indices of toxicity and their 95% confidence limits were derived:

- LC_{95} value (Lethal concentration that will cause mortality of 95% of the exposed population of test organisms)
- LC_{50} value (Lethal concentration that will cause mortality of 50% of the exposed population of test organisms)
- LC_5 value (Lethal concentration that will cause mortality of 5% of the exposed population of test organisms)

Statistical analysis: The data from micronucleus were analyzed using graphical representation, ANOVA and Duncan multiple Range test (DMRT) to test for significant difference (5% level) in the mean frequency of micronucleus induction in *Clarias gariepinus* exposed to different sub lethal concentration of spent engine oil.

RESULTS

Percentage mortality of *Clarias gariepinus* is presented in Table 1, probit of mortality against log concentration of spent engine oil against *Clarias gariepinus* at 96 h LC_{50} is presented in Fig. 1. The analysis of concentration-mortality data of spent engine oil when tested against *Clarias gariepinus* revealed that the derived toxicity indices (LC_{50}) is 2.75 (562 mL^{-1}). On the basis of computed Toxicity Factor (TF), using 96 h LC_{50}, spent lubricant oil was found to be very toxic to the *Clarias gariepinus* juveniles (Fig. 1).

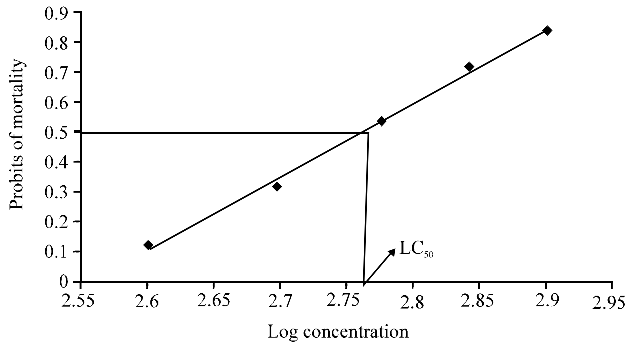

Fig. 1: Probit of mortality against log concentration of spent engine oil against *Clarias gariepinus* at 96 h LC_{50}

Table 1: Percentage mortality of *Clarias gariepinus* fingerlings exposed to spent engine oil

		Mortality (%)			
Concentration (%)	No. of organisms	24 h	48 h	72 h	96 h
Control	30	0.0^{a}	0.0^{a}	0.0^{a}	0.00^{a}
400	30	0.0^{a}	0.0^{a}	6.7^{b}	13.30^{c}
500	30	0.0^{a}	16.6^{c}	0.0^{a}	13.30^{b}
600	30	0.0^{a}	16.6^{b}	0.0^{a}	26.70^{c}
700	30	20.0^{c}	6.7^{a}	23.3^{d}	16.60^{c}
800	30	33.3^{d}	30.0^{c}	26.7^{b}	3.33^{a}

Mean values followed by same superscript in each row are not significantly different at $p<0.05$

Table 2: Mean frequencies of micronucleus induction in erythrocytes of *Clarias gariepinus* exposes to sub lethal dose of spent lubricant oil

		Engine oil conc. (mL L^{-1})		
Duration of treatment (day)	Control	100	200	300
7	10.33 ± 2.30^{a}	83.474 ± 25.84^{b}	80.00 ± 23.58^{b}	75.17 ± 21.00^{b}
14	15.50 ± 4.66^{a}	217.00 ± 60.05^{a}	180.00 ± 42.55^{b}	199.83 ± 32.18^{a}

Mean values followed by same superscript in each row are not significant different at $p<0.05$

Analysis of variance (ANOVA), showed that there was significant difference ($p<0.05$) in the quantal response (mortality) of *Clarias gariepinus* to different concentrations of spent engine oil at 24, 48, 72 and 96 h of exposure (Table 1).

Micronucleus analysis : Results reveal that the *Clarias gariepinus* specie shows various degrees of sensitivity in monitoring genetic damage (especially clastogenic effect). This is indicated by variations in averages of the micro nucleated cells among species at various test solutions. The obtained results are summarized in Table 2. The chromosomal aberrations represented by the formation of micronucleus showed marked increase in the following level of occurrences; 100, 200, 300 and 400 mL L^{-1}. Test solution of concentration 400 mL^{-1} was observed to possess fish with higher level of micronucleus frequencies.

The mean frequencies of micronucleus in *Clarias gariepinus* exposed to different concentrations of spent engine oil ranged from (10.33±230-71.54±20.01). The lowest value was 10.33±2.30 and

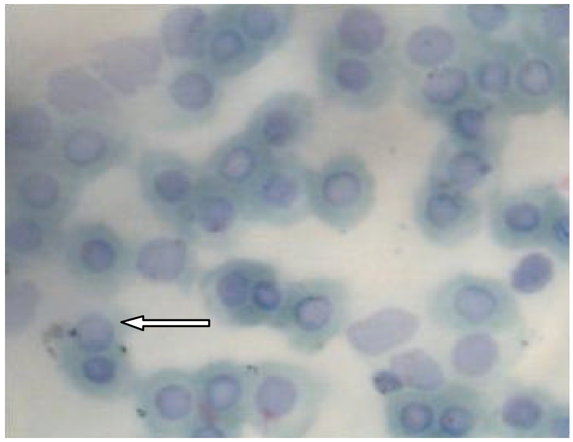

Fig. 2: Micronucleated erythrocyte (arrow) of *Clarias gariepinus* treated with 200 mL L^{-1} concentration of engine oil

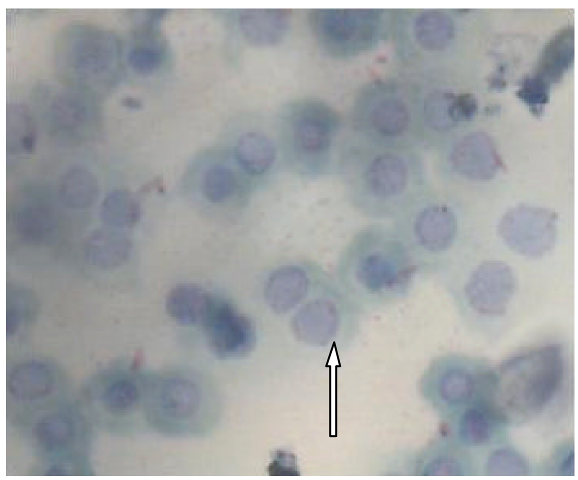

Fig. 3: Binucleated erythrocyte (arrow) of *Clarias gariepinus* treated with 400 mL L^{-1} concentration of engine oil

was recorded at day 7 in organisms exposed to control (0.00 mL L^{-1}) experiment and highest value was 71.54±20.01 and was recorded in organisms exposed to 400 mL L^{-1} test solutions. There was no significant difference in the frequencies of observed micronucleus in *Clarias gariepinus* exposed to different concentrations in day 7 and day 14 exposures. There was a significant difference in the micronucleus frequency observed between control solution and the 400 mL L^{-1} test solution.

Figure 2 shows micro nucleated cell in 200 mL L^{-1} concentration, Fig. 3 shows binucleated cell in 400 mL L^{-1} concentration, Fig 4 shows normal cell in control, Fig. 5 shows micro nucleated cell in 100 mL L^{-1} and Fig. 6 shows binucleated cell in 300 mL L^{-1} concentration in different fish *Clarias gariepinus* used for this study. Cytological examination after treatment with different doses of spent engine oil revealed that binucleated cells, deformed nuclei in addition to the main type of aberration (micronucleus) were observed. It is obvious that peripheral erythrocytes are sensitive

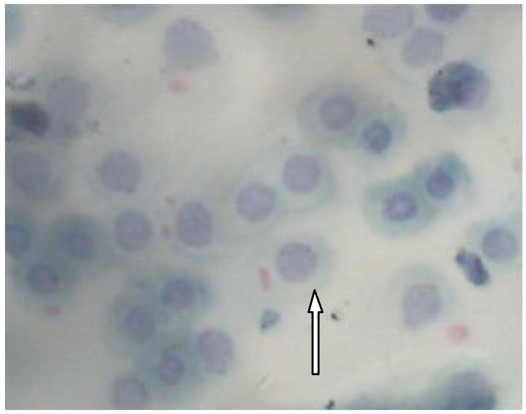

Fig. 4: Normal peripheral erythrocyte of *Clarias gariepinus* (arrow) control

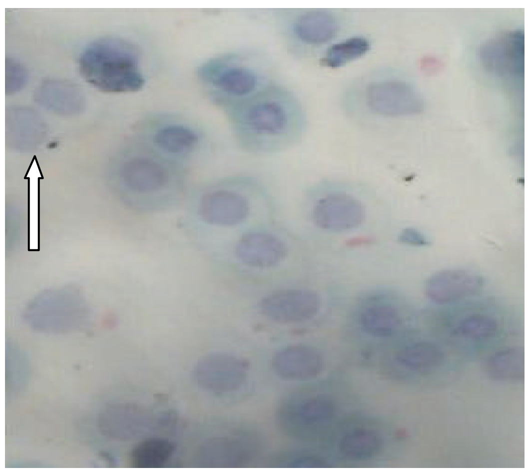

Fig. 5: Micronucleated cell (arrow) of *Clarias gariepinus* treated with 100 mL L^{-1} concentration of engine oil

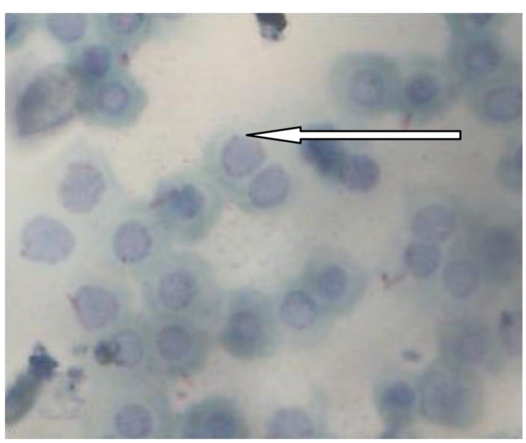

Fig. 6: Binucleated cell (arrow) of *Clarias gariepinus* treated with 300 mL L^{-1} concentration of engine oil

for the damage induced by the aquatic contaminants (approximately 150% compared with kidneys erythrocytes). The high percentage of micronuclei (MN) in peripheral blood of *Clarias gariepinus* may represent evidence that its genome well tolerates such type of cytogenetic damage without apoptosis. Peripheral blood cells were shown to be sensitive in *Clarias gariepinus* species for monitoring mutagenic and/or clastogenic effect induced by the aquatic environment.

DISCUSSION

Exposure to genotoxic agents may result in mutation, metabolic disorder, damage embryos and reduced fertility. The use of genotoxicity testing is essential for the assessment of potential livestock toxicity so that hazard can be controlled. Regulations in many countries are beginning to limit point source discharges of toxic chemicals into water sources; however, historical and current industrial and urban discharges are still responsible for high concentrations of toxic substances in aquatic environments (Rodriguez-Cea *et al.*, 2003). The results obtained from this study showed that spent engine oil was toxic to *Clarias gariepinus* with toxicity increasing with time, exposure and concentration. The 96 h Lethal Concentration (LC_{50}) obtained was similar to results obtained from Olaifa *et al.* (2004) on the lethal and sub lethal effects of copper on the African catfish (*Clarias gariepinus*). Pollution from spent engine oil is one of the growing environmental problems in Nigeria. It is pertinent to point out; however, that pollution by spent engine oil in Nigeria occurs mostly in sub lethal concentrations because the present level of spent oil in contaminated waters is not high enough to cause acute toxicity effects on aquatic pelagic and benthic organisms. This is in agreement with Russo *et al.* (2004) on contaminants present in the aquatic environment that endanger the survival and physiology of the organisms and also induces genetic alterations.

In this study, there was significant difference ($p<0.05$) between the frequencies of micronuclei obtained from the peripheral blood smears collected on day seven (7) and day fourteen (14). (Ali *et al.*, 2008) observed that *Clarias gariepinus* from freshwaters of Egypt have higher incidence of the chromosomal aberrations of micronuclei in its genome than the *Clarias gariepinus* juveniles employed in this study, maintaining the species to be highly tolerant of that particular genetic damage without triggering the genetically programmed event that allows cells to die. Hence, the statistical results of significant difference between the species of catfish from the waters of Egypt and *Clarias gariepinus* in this study might be as a result of physiological variations and responses to the local agents inducing the chromosomal damage, the micronuclei frequencies may vary according to the kind of pollution involved and the species of fish. This study indicates that nuclear abnormalities are induced in response to exposure to genotoxic agents which is similar with the observation of Malla and Ganesh (2009). Cell attachments are rarer than micro nuclei in normal cells, but can be found in up to 20% of cells treated with genotoxic substances such as spent engine oil. These attachments may result from problems in segregating tangled chromosomes. In a stress situation, erythrocyte count is one of the first parameters that are affected. In this study, it was found to increase almost immediately after the fish was transferred from control to the spent engine oil test solutions. An organism such as fish in polluted water is under stress and the extent of the stress depends on the type of pollutant and of course the amount or concentration of pollutants in the water. Most fishes have a way of detecting polluted areas but in the case where the whole water is contaminated, the fish is faced with the problem of using other means of respiration apart from the gills. In this study, low concentrations of spent engine oil do not show much increase in micronuclei or nuclear abnormalities except in high concentrations of spent engine oil. During this study, it was obvious that *Clarias gariepinus* tend to increase its atmospheric air breathing activities as the concentration of spent engine oil increased. The results of this study suggest that higher frequencies of micronuclei and nuclear abnormalities determined in the cells of *Clarias gariepinus* from the test solution may be indicative of damage caused by the pollutant in the test solution. The positive relationship between micronuclei and nuclear abnormality induction suggests that nuclear abnormalities may be useful complementary assay for genotoxicity analyses when fish are used as experimental animals. In conclusion, the findings in this study related to toxic response and sensitivity towards spent engine oil underline the need for further caution in order to ensure reduction in the risk of damage caused by multiple pollutants as they occur in ecosystems.

REFERENCES

Ali, F.K., A.M. El-Shehawi and M.A. Seehy, 2008. Micronucleus test in fish genome: A Sensitive monitor for water pollution Afr. J. Biotechnol., 7: 606-612.

Ayoola, S.O., 2008. Histopathological effects of glyphosate on juvenile African catfish (*Clarias gariepinus*). Am. Eur. J. Agric. Environ. Sci., 4: 362-367.

Campana, M.A., A.M. Panzeri, A.H. Excalante, V.J. Moreno and F.N. Dulont, 2003. Micronuclei induction in *Rana catesbeiana* tadpoles by the pyrethroid insecticide lambda-cyhalothrin. Genet. Mol. Biol., 26: 99-103.

Fleeger, J.W., K.R. Carman and R.M. Nisbeth, 2003. Indirect effects of contaminants in aquatic ecosystem. Sci. Total Environ., 317: 207-233.

Malla, T.M. and N. Ganesh, 2009. Cytogenetic and tissue toxicity by synthetic sindoor in fresh water catfish *Heteropneustes fossilis*. Biomed. Pharmacol. J., 2: 85-89.

Odiete, W.O., 2003. Standardization of test animals for toxicity evaluation for environmental regulation in Nigeria. J. Nig. Environ. Soc., 1: 340-350.

Olaifa, F.E., A.K. Olaifa and T.E. Onwude, 2004. Lethal and sub lethal effects of copper to the African catfish (*Clarias gariepinus*) Juveniles. Afr. J. Biochem. Res., 7: 65-70.

Palhares, D. and C.K. Grisolia, 2002. Comparison between the micronucleus frequencies of kidney and gill erythrocytes in tilapia fish, following mitomycin C treatment. Genet. Mol. Biol., 25: 281-284.

Rodriguez-Cea, A., F. Ayllon and E. Garcia-Vazquez, 2003. Micronucleus test in freshwater fish species: An evaluation of its sensitivity for application in field surveys. Ecotoxicol. Environ. Safety, 56: 442-448.

Russo, C., L. Rocco, M.A. Morescalchi and V. Stingo, 2004. Assessment of environmental stress by the micronucleus test and the comet assay on the genome of teleost populations from two natural environments. Ecotoxicol. Environ. Saf., 57: 168-174.