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# Genotoxic Potential Index of Some Selected Sawdusts of Different Woodtypes in African Catfish (Clarias gariepinus)

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***Abstract-*** Sawdusts are major by-product of wood processing with diverse uses. Extracts of sawdust of different wood type differ due to the different chemical composition inherent in them. The aim of this work was to utilize micronuclei test as a standard system to monitor toxicity using *C. gariepinus* as a bioindicator. For this, the fish were exposed for 21 days to extracts of five species of woodshavings /sawdusts collected from Okobaba Timber depot, Lagos. Extracts of *Populus euphratrica* oliver, *N. dendiirichi*, *Eucalyptus botryoides*, *Acacia seberiana*, *Raphia hookeri mann* were used. Micronucleus genotoxic studies were carried out at graded concentrations of the extracts. Genotoxic procedure was done through collection of blood from the peripheral caudal fin. Sublethal analysis shows that for all the test organisms, the genotoxic frequency per 1000 erythrocytes evaluated varies with range from 0 – 15, 0 – 11, 0 – 15 and 1 – 24 for AERN, AEND, AEPE, AEAS and AEEB respectively over a period of 21 days. ANOVA shows significant genotoxic difference ($P < 0.05$) in the micronuclei of *C. gariepinus* exposed to different wood extracts. Clearly, different woodtypes exude varied chemicals with attendant relative toxicity.

***Index Terms-*** Extracts, Woodshavings, Micronucleus, Genotoxic, Bioindicator

## I. INTRODUCTION

Sawdust is a by product of cutting, grinding or otherwise pulverizing wood with a saw or other tools. It is composed of fine particles of wood. Woods are of economic importance to the country thereby creating revenue and of great medical importance, help in the manufacturing industries, cosmetics, pesticides, agriculture, etc. (Atkinson 2005). Mostly in Africa, the use of trees plant basically all plant are use for the treatment of various aliment and diseases. (Kuniyin 2008). In England, cider plant has been use to manufacture vinegar for treating infectious diseases and also use as the simple antiseptic (disinfectant). (Brenda 1999). Cinchona tree specifically the leaves is used to cure malaria and stop shivering and also relaxation of muscles. Birch tree the leaves, buds, bark and sap are used for easing headache produced by allergies and for detoxifying the nervous system. Neem tree, the seed, bark and leaves contains compound with proven antiseptic, antiviral, antipyretic, and anti inflammatory, anti ulcer and anti fungi uses. (Atkinson 2005). Trees are transported in Nigeria via water course of Lagos lagoon from different state in Nigeria viz Ondo state, Osun state, Delta to Okobaba, Ebute-metta Lagos. The transportation of tree on water are of dangerous effect to the aquatic life. (Nwankwo and Okeowo 2006). Wood contains poisonous compound that are presume to protect the plants against insect, bacteria etc. which are the same time effect change the water quality, affect surface dweller organism and bottom dwellers i.e. benthic organism at any concentration depending on the organism's tolerance level (Kusemiju 2001). The location of sawmill at any point close to the lagoon posses dangerous effect on man and its environment. (Abu et al 2000). According to Nwankwo 2001, sawmill located in the western part of the Lagos lagoon at Oko-baba, Ebute–metta, present a major source point of pollution. The wood waste (sawdust, wood shavings and leacheates) are deposited on the shore of the lagoon from where they eventually find their way or drain into the lagoon which alter the aquatic food web and pose hazard to aquatic organisms.

Genotoxicity studies using cytogenetic analysis in fish have demonstrated the sensitivity of this organism (Al-sati and Metcalfe, 1995), among the currently available test organisms, the micro nucleus assay is the most widely applied method due to its proven suitability for fish species (Al-Sabti 1991). The test of genetic change widely use micro nucleus test to detect both mutagenic effect and clastogenic effect and can detect the genotoxicity of a wide range compound.

The processed bark of *Populus enphratica oliver* because of its high tannic acid, it is used for tanning leather and it is treating rheumatism and also to relieve the pain of menstrual cramps. *Nauclea diderichii* known as opepe in Nigeria i.e. cheese wood, the bark is used to treat fever and stomach problem (Brown 1978). *Eucarlyptus botryoides* known as odogbo (cider mahogany) is used for treating lungs problems and nasal congestants and anti-bacterial. *Raphia Hookerii mann* known as akun (Raphia palm) is used to treat wound and also taps palm wine which is left to ferment and then taken for easy digestion. Lastly Acacia sieberiana known as irugba (Africa locust beans) is used to treat colds, cough and child hood fever. The root is used to treat stomach ach. The bark, leaves and gums are used to treat tapeworm, bilharzias, haemorrphage, orchitis, gonorrhorrea, kidney problems, syphilis, rheumatism and disorder of circulatory system, oedemadropsy (Bamowo 2001).

The species mostly used in the present bioassay is *Clarias gariepinus* (catfish). *Clarias* sp mostly inhabit fresh water and are widely distributed in Africa and Asia *clarias gariepinus* is common in Nigeria. Catfish is available all round the pool and water logged, marshy area. *C. gariepinus* is mostly used because

of the strength it exhibit to stress and environmental condition (Akpata 2002).

According to Bamowo (2001), the micronucleus test is majorly a key role/ investigator in checking the composition of pollutant in a water body which brings about changes in genetic composition either by affecting it or by forming a normal genetic trait. In other words, the micronucleus frequency may vary according to the season, the kind of pollutant involved and the species of fish.

**Aim**:

To investigate the genotoxic potential of *P. enphratica o*liver *N. dendiirchi, E. botryoides, A. seberianai and R. hookeri* using micronucleus induction of peripheral erythrocytes from caudal fin region of *C. gariepinus*.

## II. MATERIALS AND METHOD MATERIALS

Slides
EDTA bottle
Blade
Rectangular plastic tank
Fish
Bioassay bowl
Net
Paper tape
Measuring cylinder

**Equipment**

Homogenizer
Thermometer
PH meter
Microscope
Centrifuge
Spectrophotometer
Weighing balance

**Test organisms**

The test organisms for this research are fingerlings of African catfish [Clarias *gariepinus*]. C. *gariepinus* was purchased at Oloruntobi fish farm at Ayobo Ipaja in Lagos and transported in an oxygenated gallon. C. *gariepinus* has a total length of 5-8 cm and was taken to the environmental biology laboratory of the department of biological science, Yaba College of Technology. They were acclimatized for 7 days in a big rectangular plastic tank filled with borehole water (No chlorine). Feeding was administered using coppens twice daily. Changing of water was done every day to avoid pollution and oxygen content reduction by fish exudes and food remnants.

**Test compound**

The test compound are the aqueous extract of *Populus euphratica* oliver, *Nauclea diderichii, Eucalyptus botryoides, Raphia Hookeri Mann* and *Acacia sieberiana* were obtained at Okobaba saw mill, Ebute Metta Lagos. Extraction of the aqueous from each sawdust was done separately.

**Aqueous extraction preparation**

100g of each powdered wood [sawdust] were separately soaked in litre dechlorinated water for 2 days to ferment. After the 2 days of soaking the solution was filtered using a muslin cloth to separate the aqueous extract from the residue. Then the solution was kept in plastic containers at room temperature in the laboratory.

**Genotoxic slide procedure**

Blood smear was made on a slide and allow to dry .the slide are then washed with ethanol.10% Gieshma solution was poured in a glass cup raker and the slides were arranged in it. It was left for 30mins and then washed with water. It was allow to dry and then viewed under oil immersion microscope.

**Acute toxicity bioassay**

A static bioassay procedure was adopted for all the toxicity tests. A given volume of dechlorinated water was measured using a measuring cylinder into bioassay plastic tank and a predetermined volume of aqueous extract of *Populus euphratica* oliver, *E. botryoides, Raphia hookerii mann* and *Acacia seiberiana* was added to the water to make it up to 2000ml (total volume of test media) to achieve the desired test concentration.
Ten active fishes were introduced into the test medium containing the aqueous of AEPE, AEND, AEAS, AEEB and AERH each concentration bowl and a control was also set aside.
AEPE – *Populus euphratica* oliver
AEND – *Nauclea diderichii*
AEEB – *Eucalyptus botryoides*
AERH – *Raphia hookerii mann*
AEAS – *Acacia seiberiana*

**Assessment of Quantal Response [Mortality]:** Mortality assessment was carried out every 24 hours over a 96 hours experimental period. Fish was assumed to be dead when there was no body movement, even when prodded with probe.

## III. STATISTICAL ANALYSIS

**Acute toxicity data**

The quantal response (dose-mortality response) of the 96h toxicity tests were analyze after Finney (1971). The indices of toxicity measurement derived from the analysis were;

- $LC_{50}$: the concentration that kills 50% of the population
- TF: Toxicity factor for relative potency measurements.

Analysis of variance (ANOVA) were used to test for significant differences (5% level) in the means mortality response of *C. gariepinus* to different concentration of AEPE, AERH, AEAS, AEEB, AEND at 24, 48, 72 and 96hrs of exposure. All analysis was carried out using SPSS 10.1 for windows.

**Sublethal analysis (Genetic toxicity)**

The data from micronucleus were analyzed using graphical representation, ANOVA to test for significant difference (5% level) in the mean frequency of micronucleus induction in C. *gariepinus* exposed to different sublethal concentrations.

## IV. Results and Discussion

**Physio-chemical characteristics of the test media**

The mean values for the physico-chemical parameters of the test media throughout the period of experiment were pH [7.21], dissolve Oxygen 14.34mg/l, temperature 24.5°C.

**Micronucleus induction in caudal fin erythrocytes of C. *Gariepinus***

The result of the frequencies of micronucleus in caudal fin erythrocytes *C. gariepinus* exposed to sublethal concentrations of AEAS, AERH, AEPE, AEEB and AEND presented in table. The mean frequencies of micronucleus induction in the peripheral erythrocytes of *C. gariepinus* exposed to AERH, AEPE, AEAS, AEEB and AEND range from 0.00 to 20, 1.00 to 11.00, 0.00 to 15.00,1.00 to 24, 0.00 and 20 respectively.

In *C. gariepinus* exposed to AERH, the lowest value was 0.0 was recorded at day 4,7,14 and 21 in organism exposed 0.00g/l [control], 6.0g/l, 7.0g/l 0 and 10.0g/l and the highest value was 11 was recorded at day 14 in organisms exposed to 6.0g/l.

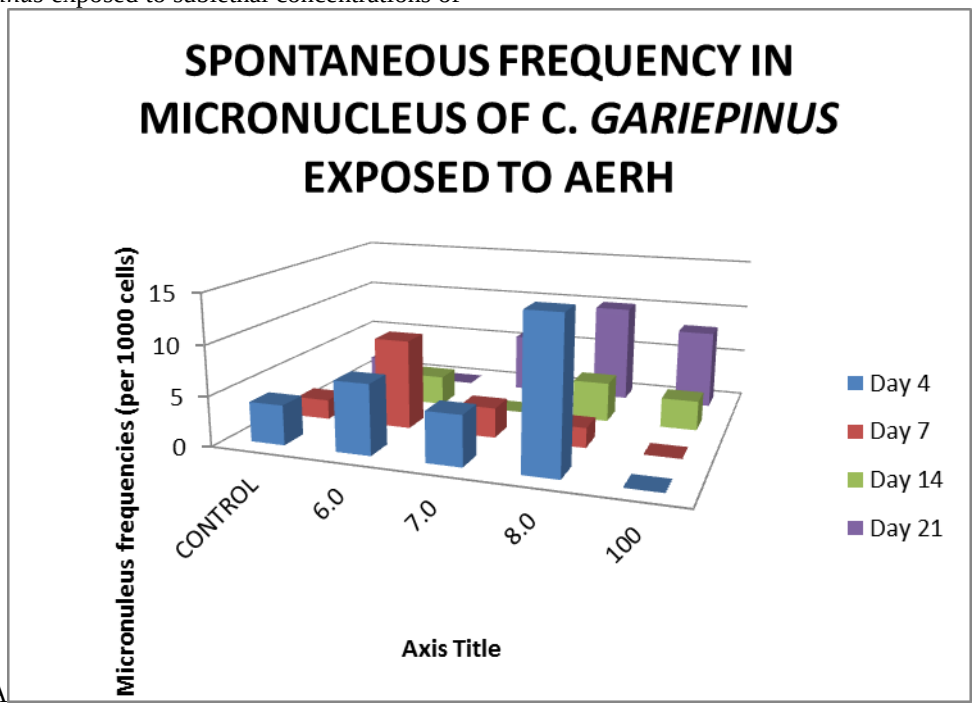


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**Figure 1: Micronucleus frequency of *C.gariepinus* expose to AERH**

Analysis of variance Analysis of variance (ANOVA) showed that there was significant difference ($P<0.05$) in the mean frequencies of micronucleus in *C. gariepinus* exposed to different concentration of AERH at day 4, 7, 14 and 21.

In C. *gariepinus* exposed to AEND, the lowest value was 0.0 was recorded at day 4,7,14 and 21 in organism exposed 0.00g/l[control],6.0g/l,8.0g/l and 10.0g/l and the highest value was 11 was recorded at day 14 in organism exposed to 6.0g/l.
Analysis of variance Analysis of variance (ANOVA) showed that there was significant difference ($P<0.05$) in the mean frequencies of micronucleus in *C. gariepinus* exposed to different concentration of AEND at day 4, 7, 14 and 21.

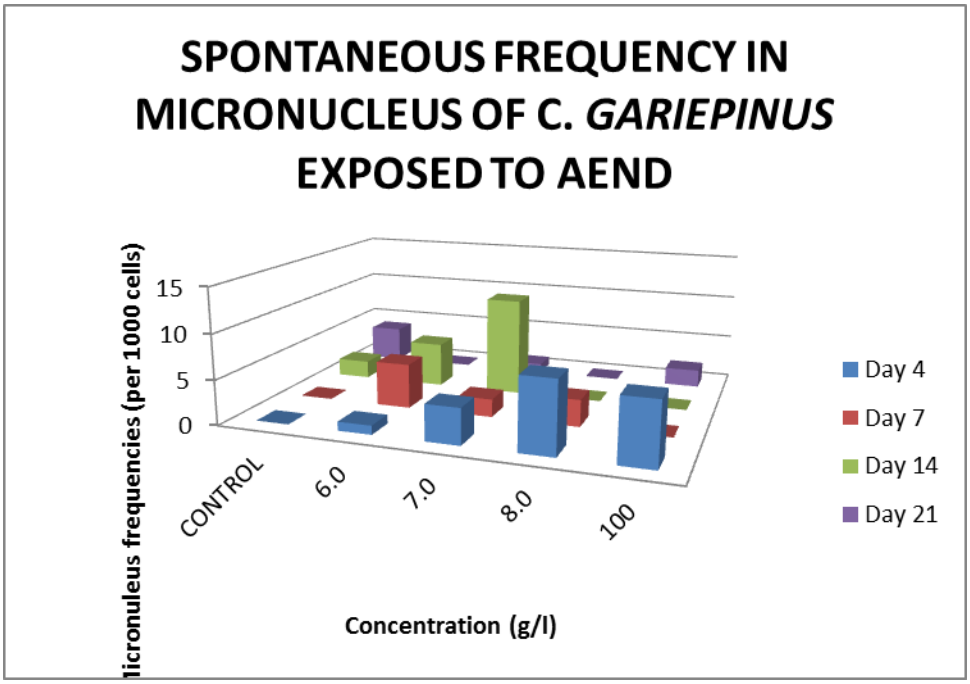


**Figure 2: Micronucleus frequency of *C.gareipinus* expose to AEND**

In *C.gariepinus* exposed to AEPE the lowest value 1.00 was revealed at day 4,14,21 in organism exposed to 0.00g/L (control), 7.0g/l, 8.0g/l and the highest value, 11 was recorded at day 14 in organisms exposed to 8.0.mg/dl

Analysis of variance Analysis of variance (ANOVA) showed that there was significant difference ($P<0.05$) in the mean frequencies of micronucleus in *C. gariepinus* exposed to different concentration of AEPE at day 4, 7, 14 and 21.

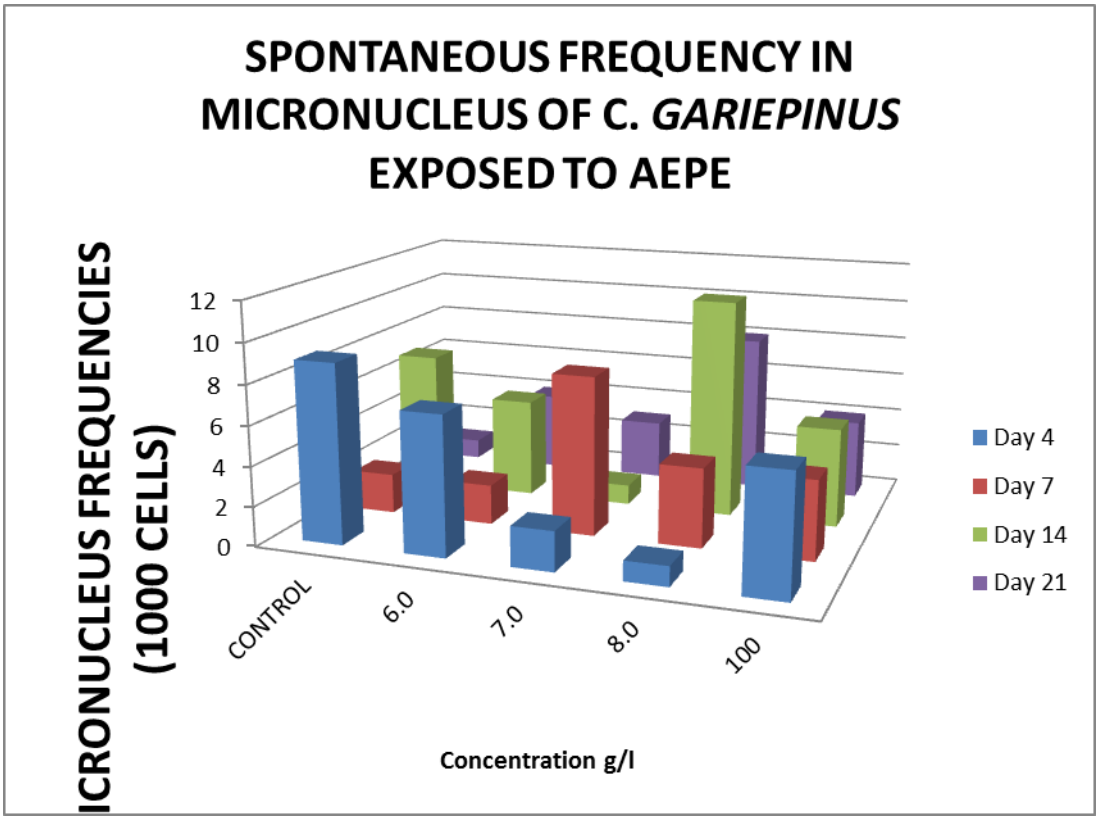


**Figure 3: Micronucleus frequency of *C. gariepinus* expose to AEPE**

In *C.gariepinus* exposed to AEEB the lowest value 0.00 was revealed at day 4, 7,14,21 in organism exposed to 0.00g/L (control),6.0g/l,7.0g /l and the highest value, 15 was recorded at day 4 in organisms exposed to 8.0.mg/dl

Analysis of variance (ANOVA) showed that there was significant difference (P<0.05) in the mean frequencies of micronucleus in *C.gariepinus* exposed to different concentration of AEEB at day 4, 7, 14 and 21.

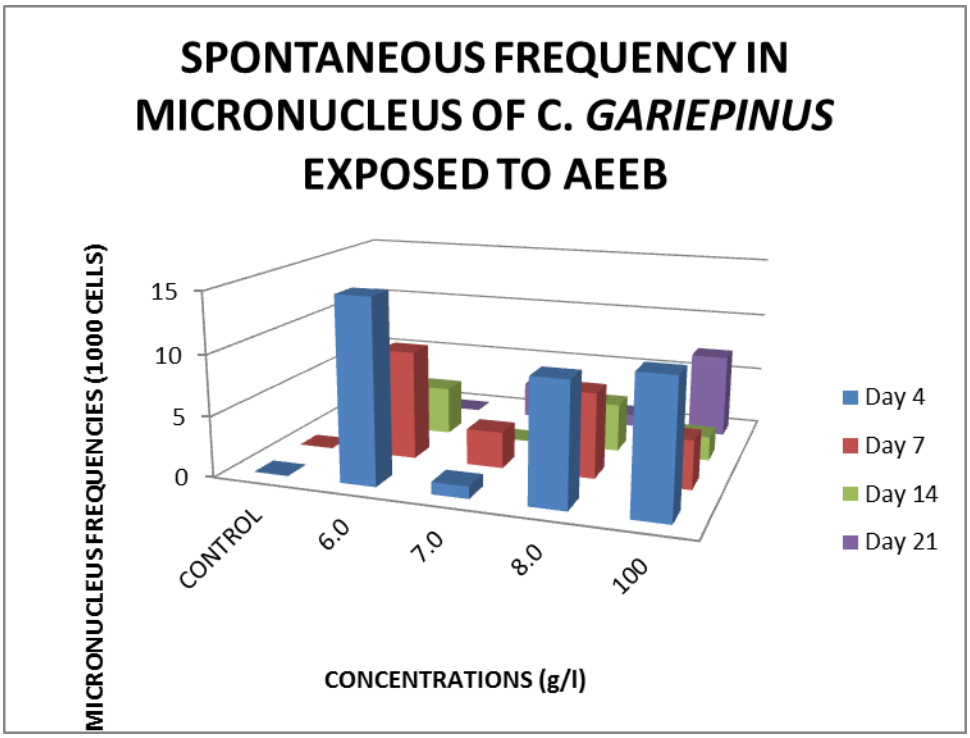


**Figure 4: Micronucleus frequency of *C.gareipinus* expose to AEEB**

In *C.gariepinus* exposed to AEAS the lowest value 1.00 was revealed at day 21 in organism exposed to 0.00g/L (control),6.0g/l,8.0g /l and the highest value, 24 was recorded at day 4 in organisms exposed to 8.0.mg/dl.

Analysis of variance (ANOVA) showed that there was significant difference (P<0.05) in the mean frequencies of micronucleus in *C.gariepinus* exposed to different concentration of AEAS at day 4, 7, 14 and 21.

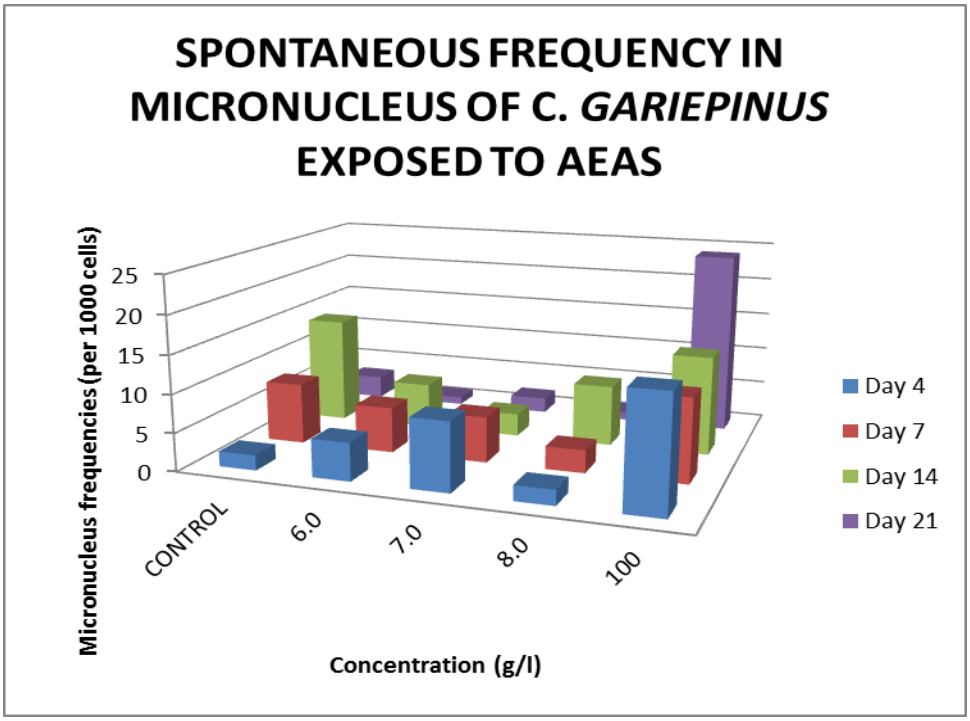


**Figure 5: Micronucleus frequency of *C. gariepinus* expose to AEAS**

The reaction of *C. gariepinus* to sublethal percentage of *Populus enphratica* oliver increase with day and the micronucleus increases with increasing exposure (Bamowo, 2011). The evaluation of toxins by genotoxicity assays provides useful data for hazard identification and comparative risk assessment (Claxton et al., 1998). In this study, erythrocytes were collected by caudal puncture of the *C. gariepinus* and this was done after one 7 days of acclimatization, and 4 days, 7 days, 14 days and 21 days of exposure. Little resistance to stress were observed during blood collection as against reports on Tilapia species by Lemos et al (2005).

Akinsan (1999), Kligerman (1982) demonstrated that fish that inhibit polluted waters have greater frequency of micronuclei.

Conclusively, the rate of DNA damage is a function of concentration level and the degree of exposure.

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