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Textile Effluent Induced Genotoxic Effects and Oxidative Stress in *Clarias gariepinus*

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Abstract: Human and ecological disorder experienced in industrial settlements as a result of improper disposal of chemicals such as textile effluent calls for careful surveillance on the state of the environment. This study investigated the toxicity of textile effluent discharge using biochemical and cytogenetic responses to ascertain the acute and sub lethal effects on *Clarias gariepinus*. The 96 h LC_{50} of *C. gariepinus* exposed to the textile effluent was 8.203 ml L^{-1}. Fourteen day exposures to 1, 2, 4 and 6 ml L^{-1} doses were conducted and several toxicological endpoints were evaluated. Sub lethal genotoxicity and biochemical study was also carried out for fourteen days. The genotoxicity studies utilized micronucleus test while the biochemical studies quantified serum anti-oxidant status Total Protein (TP), Catalase (CAT), Superoxide Dismutase (SOD) and Malondialdehyde (MDA) of the exposed fish. Toxicity factor indicates that the 96 h LC_{50} was significantly more toxic than the 24 h LC_{50} ($p<0.05$). The textile effluent at the tested concentrations induced micronucleus and nuclear abnormalities in the peripheral blood of exposed fish. Micronucleus, notch and binucleated cell formation were significant ($p<0.05$) compared to control while lobed and blebbed cells were insignificant ($p<0.05$). SOD, TP and CAT significantly ($p<0.05$) decreased compared to control group while MDA increased compared to control but was insignificant ($p>0.05$). The results obtained from this study showed that textile effluent increase cytogenetic damage and altered anti-oxidant status in *C. gariepinus*. Chemicals in the effluent can be bioaccumulated and biomagnified in the aquatic organism hence affecting man.

Key words: Bio-indicator, textile effluent, genotoxicity, oxidative stress, lipid peroxidation, *Clarias gariepinus*, pollution

INTRODUCTION

The human and ecological disorder experienced in industrial settlements as a result of improper disposal of chemicals such as textile effluent and other pollutants call for careful surveillance on the state of the ecosystem. The release of harmful chemical compounds into the environment has the potential to disturb the physiology of aquatic organism and the environment (Ayoola and Akaeze, 2012). As industrial technology improves, the characteristics of industrial discharge sources become more complicated and the toxicity of industrial wastewater can also become more complicated and heavier. Since the aquatic environment is the ultimate recipient of the pollutants produced by natural and anthropogenic sources, accumulation and persistence industrial effluents in the aquatic environment constitute a threat to biological life (Fleeger *et al*., 2003). Industries with their operations and processes are diverse in their products. Textile industry, after manufacturing are subjected to several processes known as "finishing" and these contributed major waste of effluents produced (Nosheen *et al*., 2000). Textile effluent is a complex mixture of organic and inorganic component, able to pose a direct and often continuous input of pollutants/toxicants into aquatic ecosystems. The implication on ecosystem functioning, therefore, is to ascertain the genetic effects and biochemical change in the antioxidant enzymes from these complex mixtures in aquatic organism. Micronucleus test are usually performed using red blood cells, although liver and gill tissues are also used in animals' most especially aquatic organism like fishes (Ferraro *et al*., 2004; Beninca, 2006). Biomarkers determined the biochemical, physiological and histological alterations by the exposition to xenobiotics (Amado *et al*., 2006). Oxidative stress may ensue when the ability to buffer against Reactive Oxygen Species (ROS) is exceeded either by excessive production of ROS or by depletion of antioxidant. Aquatic organism most especially Fish can serve as bioindicators of environmental pollution and can be used for the assessment of the water quality (Dautremepuits *et al*., 2004). However, they are directly

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exposed to chemicals resulting from discharge via surface runoff of water or indirectly through the food chain of ecosystem (Lopes *et al*., 2001). Some of the aquatic organism especially fish are with defensive mechanisms to counteract the impact of reactive oxygen species which result in metabolism of various chemicals and effluents. The indiscriminate discharge of effluent into aquatic ecosystem has increased over the ages, especially in the developing countries like Nigeria (WHO, 2003); textile effluents are refer to as toxicants which result in acute disorders in animals most especially aquatic organisms. There is paucity information in the broad review of literature on the genotoxic effect using micronucleus assay and biochemical changes of antioxidant alteration in *Clarias gariepinus*. There are impairment when cellular defense systems are exposed to pollutants. However, the levels of antioxidants in living organisms may increase in order to restore the imbalance caused by oxidative damage. The changes in the antioxidant enzymes, glutathione system and induction of lipid peroxidation reflect the presence of metals which may cause oxidative stress in fish (Farombi *et al*., 2007). Therefore, this study was set to evaluate the genetic damage and biochemical displacement as a result of increased stress induced by the reactive oxygen species of the effluent.

MATERIALS AND METHODS

Test animal: The experiment was carried out between February and October 2011. Juveniles of *C. gariepinus* were used in this study for bioassays. The fish (Total length 6.6±0.74 cm and weight 9.76±0.85 g) were purchased from a reputable fish farm and transported in an oxygen bag to the laboratory at department of Marine Sciences, ecotoxicology unit, University of Lagos, Akoka, Nigeria. The *C. gariepinus* was kept in a plastic tank (34×27×48.5 cm) which was half filled with dechlorinated water. During acclimatization, the juveniles were fed with commercial fish feed which contains 35% crude protein and they were fed 5 ml L^{-1} of their body weight.

Experimental set-up: The textile effluent was obtained from a textile company through an untreated discharge source. Based on this, five concentrations (0.0, 5, 10, 15, 20 and 25 ml L^{-1}) of the textile effluent were prepared and tested on the *C. gariepinus* juveniles for the definitive test. Ten acclimated fish were used in each plastic tank containing different concentrations of textile effluent as well as in the control as describe by (Rahman *et al*., 2002). Textile effluent: 0 (control), 1, 2, 4 and 6 ml L^{-1} concentration were used for the sub lethal analysis.

Bioassay procedure: A static renewal bioassay technique was adopted in which the test media were renewed at the same concentration once every 24 h (ASTM, 1991). Preliminary screening was carried out to determine the appropriate concentration range for testing chemical (Solbe, 1995).

Physico-chemical analysis: Physico-chemical parameters of the test media were collected and analyzed during the 96hrs exposure. Physicochemical measurements of test media were observed at the beginning of the experiment and at the end (that is, before change of test media). The parameters measured are dissolved oxygen, pH and temperature using appropriate digital instruments (Horiber U-10).

Cytogenetic analysis: Peripheral blood samples were obtained from the caudal peduncle and immediately smeared. After drying for 24 h at room temperature, the smeared slides were fixed with methanol for 30 min. One thousand erythrocytes were examined per fish in coded slides set replicate of fishes per concentration; five slides were prepared from a concentration of triplicates, making two thousand scored cells per fish. The peripheral blood smears were obtained through the blood by means of a serum imprint following dissection as described by Ali *et al*. (2008) and Palhares and Grisolia (2002).

Biochemical analysis

Determination of malondialdehyde (MDA): The levels of homogenized tissue MDA, as an index of lipid peroxidation were determined by Thiobarbituric Acid Reaction (TBARS Assay) using the method of Yagi (1998).

Assay of antioxidant enzymes

Superoxide Dismutase (SOD): Superoxide Dismutase (SOD) enzyme activity was determined according to the method by Sun and Zigman (1978).

Catalase (CAT): The activity of the enzyme catalase was analyzed following the method adopted by Sinha (1972) and assayed calorimetrically at 620 nm.

Statistical analysis: Toxicological data involving quantal response (mortality) were analyzed by probit analysis after Finney (1978). One-way analysis of variance, ANOVA was used for biochemical and micronucleus test analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) were used. Values were considered significant when $p<0.05$. The data from the blood were also analyzed using graphical representatives. Analysis was performed using SPSS 18 for windows.

The indices of toxicity measurement derived from the analysis were:

LC_{50} = The concentration that kills 50% of the test population
LC_{95} = The concentration that kills 95% of the test population
TF = Toxicity factor for relative potency measurements

RESULTS

Physico-chemical parameters of textile effluent samples collected from the industrial discharge: Physicochemical measurements of test media were made at the beginning of the experiment and at the end (that is, before change of test media). The parameters measured are dissolved oxygen, pH and temperature using appropriate digital instruments (Horiber U-10).

The Physico-chemical parameters of the effluent samples used for this study are presented in Table 1. The textile effluent of pH has a mean range of 7.65±0.51 at day 2 to 9.36±0.30 at day 3 during acute toxicity testing and this result was observed to be slightly higher than the Federal Environmental Protection Agency (FEPA) limit of 6-9. The temperature values ranged from 28.39±0.21 to 28.3°C±0.98 which falls within the guideline of FEPA limit and DPR. The mean value range of dissolved oxygen ranged from 1.99 to 5.67 mg L^{-1} as represented in (Table 1). The water quality variable differs between each days of exposure of fish to different concentrations (Table 1). The pattern of behavioral responses and mortality was observed at the various concentrations. *C. gariepinus* juveniles treated with textile effluent showed initial distress swimming movements, rapid opercular and tail movements, loss of balance, incessant gulping of air, mucus secretion especially on the gills, unusual lethargy and fish settling at the bottom motionless with slow opercular movement before death.

Relative and comparative toxicity of textile effluent exposed to *C. gariepinus*: The results of the acute toxicity of textile effluent on *C. gariepinus* at 24, 48, 72 and 96 h of exposure are shown in Table 2. The analysis of concentration-mortality data of textile effluent when tested against *C. gariepinus* revealed that the derived toxicity indices (LC_{50}) ranged from 8.203 (96 h LC_{50}) to 21.581 (24 h LC_{50}) (Table 2). On the basis of computed Toxicity Factor (TF) using 96 h LC_{50}, textile effluent was found to be more toxic against *C. gariepinus* at the 96 h LC_{50} with 2.63 compared to others. In this study, the acute toxicity level based on the 96 h LC_{50} value of textile effluent was found to be 8.203 ml L^{-1} when tested against the *C. gariepinus* (Fig. 1). ANOVA showed that there was significant difference ($p<0.05$) in the quantal response at 24, 48, 72 and 96 h of exposure. This shows that the mortality of the organisms in the treatment increases as the level concentration increases and time of exposure (Table 3). The Probit analysis

Table 1: Physico-chemical characteristics of the textile effluent collected from discharge point

Parameters	Day 1	Day 2	Day 3	Day 4	Mean	Std. error	*FEPA limit
pH	8.78±0.72^{a}	7.65±0.51^{a}	9.36±0.30^{a}	8.68±0.25^{b}	8.62	0.71	6-9
Temperature (°C)	28.39±0.21^{a}	28.15±0.78^{a}	28.48±0.54^{b}	28.30±0.98^{ab}	28.33	0.14	<40
Dissolved oxygen (mg L^{-1})	5.67± 0.12^{a}	1.99±0.11^{a}	5.32±0.23^{ab}	4.87±0.32^{a}	4.46	1.68	NS

Mean±SD with the same alphabet in each exposure period are not significantly different ($p>0.05$, DMRT), *Federal environmental protection agency

Table 2: Textile effluent toxicity against *Clarias gariepinus* juveniles

Exposure time (h)	LC_{50} (95 ml L^{-1} CL ml L^{-1})	LC_{5} (95 ml L^{-1} CL ml L^{-1})	LC_{95} (95 ml L^{-1} CL ml L^{-1})	Slope±SE	Probit line equation	DF	TF
24	21.581 (23.05-20.18)	17.59 (19.12-14.22)	26.48 (32.61-24.39)	18.50±4.59	Y = -19.68+18.50x	4	1.00
48	12.519 (00.00-00.00)	7.43 (0.00-00.0)	21.09 (0)	7.27±1.192	Y = -2.97+7.27x	4	1.72
72	9.050 (10.60-7.44)	4.01 (5.28-2.44)	20.43 (29.07-16.58)	4.65±0.754	Y = 0.55+4.65x	4	2.38
96	8.203 (9.53-6.85)	4.15 (5.27-2.69)	16.22 (22.41-13.39)	5.56±0.924	Y = -0.08+5.56x	4	2.63

CL: Confidence limit, SE: Standard error, DF: Degree of freedom, LC: Lethal Concentration, TF: Toxicity factor:

$$\text{Toxicity factor} = \frac{LC_{50} \text{ of test compound at 24 h}}{\text{Test compound at other hours (48, 72, 96h)}}$$

Probit line equation (y) = a +b log C, LC: Lethal concentration

Table 3: Percentage mortality of *Clarias gariepinus* juveniles exposed to textile effluent

		Mortality (%) after			
Concentration (ml L^{-1})	No. of test organisms	24 h	48 h	72 h	96 h
Control (0)	21	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
5	21	0.0^{a}	4.8^{a}	19^{ab}	19^{a}
10	21	0.0^{a}	9.5^{a}	42.9^{ab}	47.6^{a}
15	21	0.0^{a}	66.7^{b}	81^{b}	100^{b}
20	21	9.5^{a}	100^{c}	100^{c}	100^{c}
25	21	100^{b}	100^{c}	100^{c}	100^{c}

Percentage mortality with time (hours) with the same alphabet in each exposure period are not significantly different ($p>0.05$, DMRT)

showing the log concentration plotted against the probit percentage mortality of the fish exposed to textile effluent was represented in Fig. 1. No adverse behavioural changes or any mortality were recorded in the control fish throughout the period of the bioassay. The behaviour of the control fishes and their colour were normal. Symptoms of toxicosis observed in fish behaviour with textile effluent include lack of balance; agitated or erratic swimming, air gulping, restlessness, sudden quick movement, excessive secretion of mucus, rolling movement and swimming on the back were observed. The exposed fish became very weak, settled at the bottom and died and the skin colour was shining.

Micronucleus analysis: The mean frequencies of micronucleus (MN) and nuclear abnormalities (Binucleated (BN), Notched (NT), Lobed (LB) and Blebbed (BL)) in *C. gariepinus* exposed to different concentration of textile effluent ranged are presented in

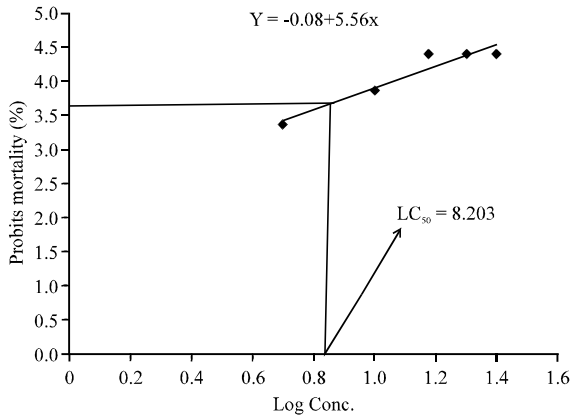

Fig. 1: Lethal concentration (LC_{50}) of *Clarias gariepinus* exposed to textile effluent

Table 4. The lowest value was recorded at day 3 in organism exposed to control (0 ml L^{-1}) experiment and highest value was 9.33 which was recorded in organisms exposed to 6 ml L^{-1} at day 7 for micro nucleated cells. For micronucleus analysis, the mean frequencies of micronucleus in *C. gariepinus* exposed to different concentrations of the effluent showed that there is a significant difference ($p<0.05$) (Table 4). ANOVA (DMRT) showed that there was a significant difference ($p<0.05$) in the frequencies observed for micro nucleated cells in *C. gariepinus* exposed to 6 ml L^{-1} and control (0 ml L^{-1}) concentrations at day 3 while at day 7 there was also a significant difference ($p<0.05$) observed in 1 and 4 ml L^{-1} concentrations. Furthermore ANOVA showed that there was significant difference ($p<0.05$) in 1, 2 and 6 ml L^{-1} concentrations at day 14 (Table 4).

Generally, binucleated cells which indicate a significant difference ($p<0.05$) at day 3 and 7 while at day 14, there is no significant difference ($p>0.05$). For Notched cell, there is a significant difference ($p<0.05$) at day 3, 7 and 14. For Lobed cells, there was no significant difference ($p>0.05$) at day 3, 7 and 14. While for blebbed cells, there was a significant difference ($p<0.05$) at day 7and no significant difference ($p>0.05$) at day 3 and 14 (Table 4). However, the treatments with textile effluent significantly induced the formation of nuclear abnormalities BN, LB, BL and NT especially at the highest doses (Table 4). ANOVA using the DMRT post-hoc method, showed that there is no significant difference ($p>0.05$) in BN, LB, BL and NT frequencies observed of *C. gariepinus* exposed to concentrations of 2, 4 and 6 ml L^{-1} at day 3, 7 and 14 (Table 4).

Photomicrographs of micronucleated and nuclear abnormailiries: The photomicrograph represented in Fig. 2 show the cytogenotoxic damage impacted on

Table 4: Frequencies of micronucleus and nuclear abnormalities in blood of *C. gariepinus* exposed to sub lethal concentrations of textile effluent

	Textile effluents of micronucleus (v/v)				
Duration of treatment (day)	Control	1 ml L^{-1}	2 ml L^{-1}	4 ml L^{-1}	6 ml L^{-1}
3	1.47±0.34^{a}	3.07±0.45^{a}	7.27±1.43^{a}	4.00±1.02^{ab}	7.80±1.35^{abc}
7	1.47±0.29^{a}	7.20±1.17^{ab}	7.47±2.01^{a}	7.93±1.44^{ab}	9.33±1.81^{ab}
14	1.33±0.35^{a}	8.26±1.67^{abc}	5.80±1.22^{ab}	7.93±1.31^{a}	8.67±1.44^{ab}
Binucleated					
3	2.07±0.49^{a}	4.40±1.09^{a}	5.40±0.84^{a}	1.80±0.39^{a}	5.20±1.00^{a}
7	2.73±0.52^{abc}	3.93±0.67^{a}	2.33±0.57^{a}	5.00±1.15^{a}	5.13±1.15^{ab}
14	3.67±0.57^{ab}	7.47±1.42^{ab}	3.13±0.47^{ab}	4.67±1.04^{ab}	6.33±0.95^{a}
Notch					
3	0.40±0.16^{a}	2.20±0.66^{ab}	1.80±0.39^{abc}	1.93±0.51^{ab}	1.00±0.24^{a}
7	0.47±0.17^{a}	7.27±1.91^{ab}	1.67±0.30^{a}	1.80±0.39^{abc}	1.47±0.29^{a}
14	0.80±0.24^{a}	2.20±0.66^{ab}	0.73±0.18^{a}	1.67±0.30^{a}	1.80±0.39^{abc}
Lobed					
3	0.47±0.17^{a}	1.20±0.37^{ab}	1.93±0.51^{a}	0.73±0.18^{a}	1.93±0.49^{a}
7	0.60±0.24^{a}	1.93±0.51^{a}	1.67±0.44^{a}	1.93±0.51^{a}	1.67±0.43^{ab}
14	0.80±0.26^{a}	1.20±0.37^{ab}	1.13±0.26^{a}	1.67±0.44^{ab}	1.93±0.51^{a}
Blebbed					
3	0.40±0.16^{a}	0.87±0.22^{a}	0.73±0.81^{a}	1.00±0.28^{a}	0.80±0.22^{a}
7	0.53±0.19^{a}	5.93±1.52^{a}	1.13±0.27^{a}	0.27±0.12^{a}	0.73±0.18^{a}
14	0.67±0.23^{a}	0.87±0.22^{a}	0.53±0.17^{a}	0.87±0.22^{a}	1.00±0.24^{ab}

Mean frequencies (Mean±SE, standard error) with the superscript letter in a row are not significantly different in the DMRT (p = 0.05)

Table 5: Total protein, malondialdehyde, catalase and superoxide dismutase contents of *C. gariepinus* exposed to sub lethal concentrations of textile effluent

Concentration (mL)	TP (IU mg^{-1} protein)	CAT (IU mg^{-1} protein)	MDA (nmol mg^{-1})	SOD (IU mg^{-1} protein)
0	47.01± 2.32^{a}	57.21±18.31^{a}	2.92±0.47^{ab}	13.07±1.09^{ab}
1	35.56±5.05^{a}	82.30±29.71^{a}	5.21±2.47^{ab}	16.93±1.97^{b}
2	46.82±0.30^{a}	39.41±27.51^{a}	1.26±0.56^{a}	13.13±0.09^{ab}
4	42.72±2.90^{a}	63.24±19.79^{a}	7.05 ±1.44^{b}	14.77±0.73^{b}
6	47.87±2.03^{a}	31.49±15.62^{a}	3.27±0.75^{ab}	10.75±1.19^{a}

Mean±SE with the same alphabet in each exposure period are not significantly different (p>0.05, DMRT)

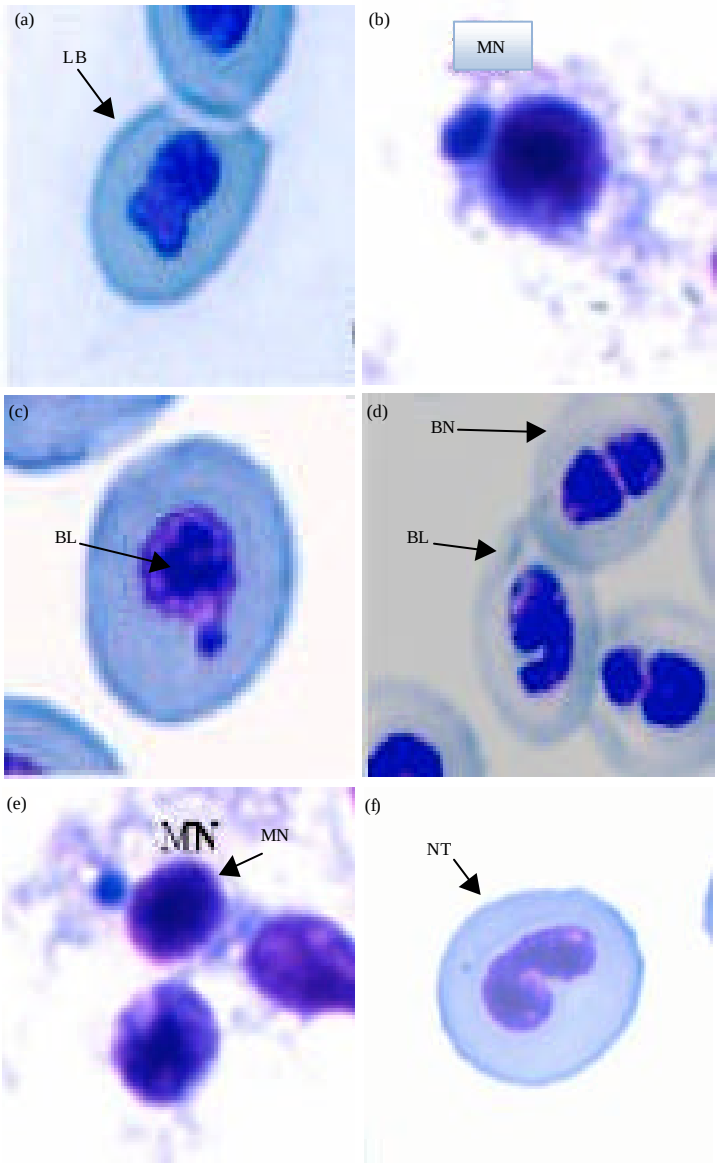

Fig. 2(a-f): Photomicrographs of micronucleus and nuclear abnormalities, (a) Lobed cell (LB) observed in concentration 6 ml L^{-1} at day 7 (b) Micro nucleated cell (MN) observed in concentration 1 ml L^{-1} at day 3, (c) Blebbed cell (BL) observed in concentration 4 ml L^{-1} at day 14, (d) Binucleated cell (BN) and Blebbed cell observed in concentration 2 ml L^{-1} at day 3, (e) Micro nucleated cell (MN) observed in concentration 1, 2 4 and 6 ml L^{-1} at day 3 and (f) Notched cell (NT) observed in concentration 4 ml L^{-1} at day 7 in blood of *C. gariepinus* exposed to sub lethal concentrations of textile effluent

nucleus of *C. gariepinus* during the fourteen day exposure to sub lethal concentrations of textile effluent. The observations made are stated below:

Biochemical parameters: The results of the biochemical changes (MDA, Superoxide dismutase and CAT) observed in the blood of *C. gariepinus* exposed to different concentrations of textile effluent is shown in Table 5. ANOVA results of the lipid peroxidation assay indicates that the level of MDA in the blood of the fish exposed to textile effluents shows no significant difference ($p>0.05$) when compared to control animals. There is no significant different ($p>0.05$) at concentration 2 and 4 ml L^{-1}. Furthermore, Post hoc using (Duncan Multiple Ranged Test) DMRT revealed that there was a significant difference ($p<0.05$) in the MDA of the blood of *C. gariepinus* exposed to 1, 6 mL and control (Table 5). The mean superoxide dismutase in the blood of *C. gariepinus* exposed to different concentrations of textile effluent ranged from 10.75±1.19 to 16.93±1.97 IU mg^{-1} proteins (Table 5). On exposure to textile effluent, *C. gariepinus* lowest mean value for superoxide dismutase was recorded at concentration of 6 ml L^{-1} while the highest mean superoxide dismutase was recorded in organism at concentration 1 ml L^{-1}. ANOVA showed that there is a significant difference ($p<0.05$) observed in the superoxide dismutase content of *C. gariepinus* exposed to all different concentrations at day 14. Furthermore, ANOVA (DMRT) revealed that there was no significant difference ($p>0.05$) at the concentration of 6 ml L^{-1} in the organisms exposed. The mean Catalase content in the blood of *C. gariepinus* exposed to different concentrations of textile effluent ranged from 31.49±15.62 to 82.30±29.71 IU mg^{-1} protein, respectively. On exposure to textile effluent, *C. gariepinus* lowest mean value for Catalase was recorded at concentration of 6 ml L^{-1} while the highest mean catalase was recorded in organism at concentration 1 ml L^{-1} (Table 5). ANOVA showed that there is a no significant difference ($p>0.05$) observed in the catalase content of *C. gariepinus* exposed to all different concentrations at day 14. Furthermore, (Duncan Multiple Ranged Test) DMRT revealed that there was no significant difference ($p>0.05$) at the concentration at all different concentrations in the organisms exposed. The mean total protein content in the blood of *C. gariepinus* exposed to different concentrations of textile effluent ranged from 35.56±5.05 to 47.87±2.03 IU mg^{-1} proteins, respectively. On exposure to textile effluent, *C. gariepinus* lowest mean value for total protein was recorded at concentration of 1 ml L^{-1} while the highest mean total protein was recorded in organism at concentration 6 ml L^{-1}.

DISCUSSION

The acute and sub lethal biochemical response and cytogenetic effect of *Clarias gariepinus* exposed to textile effluent were evaluated for 96 h LC_{50} and a sub lethal concentrations for a period of 14 days. The acute toxicity level of textile effluent when tested against *C. gariepinus* was 8.230 ml L^{-1} which was found to increase in toxicity factor as the concentration increased. Analysis of variance showed that there was a significant difference ($p<0.05$) in the quantal response of *C. gariepinus* exposed to varying concentrations. The toxicity of the textile effluent against the test organism *C. gariepinus* was found to increase with time of exposure. This is in agreement with Chukwu and Ogunmodele (2005) who studies the toxicity of industrial effluents on *Clibanarius africanus* and *Tympanotomus fuscatus*. The physico-chemical parameter for pH value falls within the FEPA limit range for industrial discharge while the mean value for water temperature was 28.3°C±0.14 which also falls below 40°C of FEPA's discharge limit range. Dissolved oxygen has a mean value of 4.46±1.68 mg L^{-1}. It is important to note that the pH of any water body is dependent on its temperature. Temperature affects physical, chemical and biological processes in water bodies and therefore, the concentration of many variables. According to Chapman and Kimstach (1992), increased temperature increases the rate of chemical reactions and decreases the solubility of gases (especially oxygen) in water. Respiration rates of aquatic organisms increase leading to increased oxygen consumption and increased decomposition of organic matter. In this study, the low level dissolved oxygen was in agreement with the findings of WHO (2000), stated that low DO content could lead to anaerobic organisms taking over with the resultant creation of conditions making the water body uninhabitable to gill-breathing aquatic organisms. Hydrogen sulphide is formed under conditions of deficient oxygen in the presence of organic materials and sulphate. These observations are in accordance with the results of Junkins (1982) who reported that the textile wastes are highly alkaline. pH of effluents affects physico-chemical properties of water which in turn adversely affects aquatic life, plants and humans. This also changes soil permeability which results in polluting underground resources of water (Rump and Krist, 1992). According to Odeigah *et al.* (1997), the impact of genotoxic wastewater on the environment and the significance to human health are difficult to predict, because wastewater are complex mixtures of chemical substances. Complete interpretation of their effect often requires, in addition chemical analysis of the constituents

that may indicate the components of the wastewater that can persist and accumulate in exposed biota and thus potentially pose a hazard to human health. The use of aquatic organism can therefore be a good model to study responses to various environmental contaminants.

Industrial waste such as textile effluent and poor water quality may results to oxidative stress and genetic damage of cells in fish. Behavioral alterations have been established as sensitive indicators of chemically induced stress in aquatic organisms. Behavioral alterations like erratic swimming, restlessness and surfacing, observed in present study when exposed to industrial effluents as also observed by Mohi-ud-Din Malla *et al.* (2011).

In this study, the micronucleus observed supported the findings demonstrated by Ali *et al.* (2008) that fish inhabiting polluted waters have greater frequencies of micronuclei. The micronuclei frequencies may vary according to the season, the kind of pollution involved and the species of fish. Therefore, it is suggested that micronuclei tests in fish erythrocytes be carried out at various times following treatments, thus making it possible to follow-up the changing micronuclei frequencies. Studies of the micronuclei rates of various fish species showed that they generally peaked between the first and fifth days after treatment (Grisolia and Cordeiro, 2000). Generally, the frequencies of micronucleus cells indicates that there was significant difference ($p<0.05$) observed at day 3, 7 and 14 in *C. gariepinus* exposed to different concentrations while the mean frequencies for notched cells also showed a significant difference ($p<0.05$) on day 3, 7 and 14. This is in accordance with the work of Mahmoud *et al.* (2010) on *O. niloticus* and *T. zillii*, indicated that, there was a significant difference between all polluted areas and the control area. These chromosomal aberrations may lead to changes in the genetic component and concern has been expressed about "genetic consequences of pollution to fish populations exposed to low levels of pollution over prolonged periods. Assessment of environmental genotoxicity revealed highest frequencies of MN and nuclear abnormalities (blebbed, notched and lobed nuclei and binucleated cells) in peripheral blood erythrocytes contaminated by different concentrations of textile effluent and this is in accordance with the findings of Cavas and Ergene-Gozukara (2005) who exposed *Mugil cephalus* to industrial effluents and aromatic hydrocarbons. Biochemical analysis investigation on the total protein concentrations in fish exposed to 2, 4 and 6.00 ml L^{-1} textile effluent respectively, showed marked elevation when compared with those exposed to 1.00 ml L^{-1} and control (0 ml L^{-1}). There was no significant difference ($p>0.05$) in the total protein concentrations of fish exposed to all different concentration of the effluent. This is in agreement with Wegwu and Omeodu (2010), who stated that the Total protein concentrations in fish exposed to 10.0 and 5.00 ml L^{-1} Aqueous Extract (AE), respectively, showed marked elevation when compared with those exposed to 1.00 ml L^{-1} AE and the control fish (0.00 ml L^{-1} AE). There was no significant difference ($p = 0.05$) in the total protein concentrations of fish exposed to 1.00 ml L^{-1} AE and control. Significant decrease in total protein content indicates that, stress due to effluent treatment induces proteolysis. Stress has been reported to accelerate protein metabolism in man and animals. Protein decrease may be due to stress in fish as protein is likely to undergo hydrolysis and oxidation through TCA cycle to meet the increased demand for energy caused by the stress (Somnath, 1991). Chronic toxicants cause severe damage to the branchial system of fish than short term treatment (Chezhian *et al.*, 2010).

The mean of Malondialdehyde (MDA) showed that there is no significant difference ($p>0.05$) but has variability in values through different concentrations exposed to test organisms. The activity of antioxidant enzymes may be enhanced or inhibited under chemical stress depending on the intensity and the duration of the stress applied, as well as, the susceptibility of the exposed species.

The increase in Lipid Peroxidation (LPO) is due to an inhibitory effect on mitochondrial electron transport system leading to stimulation in the production of intracellular reactive oxygen species (Stohs *et al.*, 2000). Elevated ROS level in tissues leads to cellular damage when the rate of its generation surpasses the rate of its decomposition by antioxidant defense systems.

Increase in the activity of Catalase (CAT) and Superoxide dismutase is usually observed in the face of environmental pollutants since Superoxide Dismutase (SOD)-CAT system represents the first line of defense against oxidative stress. However, decreased CAT activity may be due to the flux of superoxide radicals which have been shown to inhibit CAT activity. Similar observation of a decrease in CAT activity following an inhibition of the activity of enzyme SOD has been reported by Fatima and Ahmad (2005).

In this study, superoxide dismutase showed a reduction in its mean values as the concentration increases indicating there was a significant difference ($p>0.05$) resulting to an inhibition in enzyme SOD in the blood of the exposed *C. gariepinus*. Superoxide dismutase catalytically scavenges superoxide radical which appears to be an important agent of toxicity of oxygen and this provides a defense against this aspect of oxygen toxicity.

CONCLUSION

The elevated levels of the biochemical parameters in this study indicate that exposure of fish to different concentrations of textile effluent would induce acute or chronic stress in fish. It is also evident from this finding that exposure of test fish to textile effluent would lead to cytogenetic damage, resulting to breakage of nucleus to form micronucleus and other forms of nuclear abnormalities or death of cells. The observed inhibition of the antioxidants defense enzymes such as SOD and CAT, in conjunction with an increase in MDA levels in the gill blood of test animals exposed to textile effluent can therefore serve as a good aid of biomarkers for early detection of pollution associated with industrial waste discharge and their inclusion in monitoring programmes are recommended. The impacts of textile effluent on the aquatic organism could be more disastrous (since it is the final receiving point of the effluents) if adequate measures are not taken before the final discharge to ameliorate the impact.

REFERENCES

ASTM, 1991. Guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. ASTM Standard Methods, 11.04, Method Number E-1367-90, American Society for Testing and Materials, Philadelphia.

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.

Amado, L.L., C.E. da Rosa, A.M. Leite, L. Moraes and W.V. Pires *et al*., 2006. Biomarkers in croakers *Micropogonias furnieri* (Teleostei: Sciaenidae) from polluted and non-polluted areas from the Patos Lagoon estuary (Southern Brazil): Evidences of genotoxic and immunological effects. Mar. Pollut. Bull., 52: 199-206.

Ayoola, S.O. and C.O. Akaeze, 2012. Genotoxic evaluation and toxicity of spent engine oil on *Clarias gariepinus*. Res. J. Environ. Toxicol., 6: 133-141.

Beninca, C., 2006. Estuarine of ponds biomonitoring camacho-jaguariuna (SC) and santa marta-laguna (SC) using geophagus brasiliensis (Cichlidae). Masters Thesis, Department of Genetics: University of Parana, Brazil.

Cavas, T. and S. Ergene-Gozukara, 2005. Micronucleus test in fish cells: A bioassay for in situ monitoring of genotoxic pollution in the marine environment. Environ. Mol. Mutagenesis, 46: 64-70.

Chapman, D. and V. Kimstach, 1992. The Selective of Water Quality Variable. In: Water Quality Assessment, Chapman, D. (Ed.). Chapman and Hall Ltd., London, pp: 51-119.

Chezhian, A., N. Kabilan, T.S. Kumar, D. Senthamilselvan and K. Sivakumari, 2010. Impact of common mixed effluent of sipcot industrial estate on histopathological and biochemical changes in estuarine fish lates calcarifer. Curr. Res. J. Biol. Sci., 2: 201-209.

Chukwu, L.O. and O.A. Ogunmodele, 2005. Toxicological response and sensitivity of estuarine macro-invertebrate exposed to industrial effluents. J. Environ. Biol., 26: 323-327.

Dautremepuits, C., S. Paris-Palacios, S. Betoulle and G. Vernet, 2004. Modulation in hepatic and head kidney parameters of Carp (*Cyprinus carpio* L.,) induced by copper and chitosan. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 137: 325-333.

Farombi, E.O., O.A. Adelowo and Y.R. Ajimoko, 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. Int. J. Environ. Res. Public Health, 4: 158-165.

Fatima, R.A. and M. Ahmad, 2005. Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in wastewater. Sci. Total Environ., 346: 256-273.

Ferraro, M.V.M., A.S. Fenocchio, M.S. Mantovani, C. de Oliveira Ribeiro and M.M. Cestari, 2004. Mutagenic effects of tributyltin and inorganic lead (P BII) on the fish *H. malabaricus* as evaluated using the comet assay and the piscine micronucleus and chromosome aberration tests. Genet. Mol. Biol., 27: 103-107.

Finney, D.J., 1978. Statistical Method in Biological Assay. 3rd Edn., Charles Griffin Ltd., London.

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

Grisolia, C.K. and C.M.T. Cordeiro, 2000. Variability in micronucleus induction with different mutagens applied to several species of fish. Genet. Mol. Biol., 23: 235-239.

Junkins, R., 1982. Pretreatment of textile waste water. Proceedings of the 37th Industrial Waste Conference, May 11-13, 1982, Purdue University, West Lafayette, Indiana, pp: 139-142.

Lopes, P.A., T. Pinheiro, M.C. Santos, M. da Luz Mathias, M.J. Collares-Pereira and A.M. Viegas-Crespo, 2001. Response of antioxidant enzymes in freshwater fish populations (*Leuciscus alburnoides* complex) to inorganic pollutants exposure. Sci. Total Environ., 280: 153-163.

Mahmoud, A., M. Zowail, G. Yossif and K. Sharafeldin, 2010. Cytogenetical studies on some River Nile species from polluted and nonpolluted Aquatic habitats. Egypt. Acad. J. Biol. Sci., 2: 1-8.

Mohi-ud-Din Malla, T., C.S. Senthilkumar, S. Akhtar and N. Ganesh, 2011. Micronuclei as an evidence of DNA damage in fresh water catfish Heteropneustes fossils (Bloch) exposed to synthetic sindoor. ARPN J. Agric. Biol. Sci., 6: 41-44.

Nosheen, S., H. Nawaz and Khalil-Ur-Rehman, 2000. Physico-chemical characterization of effluents of local textile industries of Faisalabad-Pakistan. Int. J. Agric. Biol., 3: 232-233.

Odeigah, P.G.C., O. Nurudeen and O.O. Amund, 1997. Genotoxicity of oil field wastewater in Nigeria. Hereditas, 126: 161-168.

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.

Rahman, K.S.M., T. Rahman, P. Lakshmanaperumalsamy and I.M. Banat, 2002. Occurrence of crude oil degrading bacteria in gasoline and diesel station soils. J. Basic. Microbiol., 42: 284-291.

Rump, H.H. and K. Krist, 1992. Laboratory Manual for the Examination of Water, Waste Water and Soil. 2nd Edn., VCH Publishers, New York, USA., Pages: 321.

Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem., 47: 389-394.

Solbe, J.F., 1995. Fresh Water. In: Handbook of Ecotoxicology, Calins, P. (Ed.). Black Well Science Ltd., New York.

Somnath, B., 1991. Effect of acute and sub lethal concentration of tannic acid on the protein, carbohydrate and lipid levels in the tissue of the fish Labeo rohita. J. Environ. Biol., 12: 107-112.

Stohs, S.J., D. Bagchi, E. Hassoun and M. Bagchi, 2000. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol., 19: 201-213.

Sun, M. and S. Zigman, 1978. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Anal. Biochem., 247: 81-89.

WHO, 2000. Health system: Improving performance. The world health report. Pages: 230.

WHO, 2003. The World Health Report 2003: Shaping the Future. World Health Organization, Geneva, Switzerland, ISBN-13: 9789241562430, Pages: 193.

Wegwu, M.O. and S.I. Omeodu, 2010. Evaluation of selected biochemical indices in *Clarias gariepinus* exposed to aqueous extract of Nigerian crude Oil 15. (Bonny Light). J. Applied Sci. Environ. Manage., 14: 77-81.

Yagi, K., 1998. Simple procedure for specific enzyme of lipid hydroperoxides in serum or plasma. Methods Mol. Biol., 108: 107-110.