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Evaluation of genotoxicity of a municipal landfill leachate by micronucleus test using *Clarias gariepinus*

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Abstract: The genotoxic effect of a municipal landfill leachate was evaluated in *Clarias gariepinus* using the micronucleus test. Fish was exposed to (4.95, 7.42, 14.83 and 29.66% concentration) of the leachate for 28 days and micronucleus analysis performed on peripheral blood erythrocytes. Binucleated and immature erythrocyte formations were considered along with micronuclei as cytogenetic damage indicators. The leachate induced a significant ($p<0.05$ and $p<0.001$) concentration dependent increase in micronucleus, binucleated and immature erythrocytes in *C. gariepinus*. The constituents of the leachate especially the metals were believed to cause the observed cytogenotoxic effects. This implies that solid waste leachate contains constituent capable of altering the genetic make up of aquatic forms and may predispose them to chromosome related disorders. These findings suggest that landfill leachate is capable of polluting the environment and may cause harm to public health if exposed to chemicals in landfill leachates.

Key words: Landfill leachate, Genotoxicity, Micronucleus, Erythrocytes, Cytotoxicity, *Clarias gariepinus*

Introduction

Aquatic pollution by xenobiotics has become a major problem in Nigeria, especially in Lagos State with water as its most significant topographical feature. Iwugo *et al*. (2003) reported that water and wetlands cover over 40% of the total land area of the state and most land has elevation of less than 15 m above the sea level. It is undoubtedly the commercial nerve-centre of Nigeria with the largest concentration of industrial and commercial establishments. Though, the sixth largest city in the world, Lagos state is physically small and is inhabited by a population of between 12 and 18 million people (Lawrence *et al*., 2007). These features are responsible for the daily per capital generation of 0.50 kg of municipal solid waste (MSW) in the State (Lasisi, 2001). The common methods of managing solid wastes in Lagos state is dumping in open spaces along major roads, river banks and landfilling. Landfills in the state are unlined, lack landfill caps and leachate collection systems. They are usually sited in public locations surrounded by residential structures, and this may predispose living organisms to hazardous chemicals present in landfill gas and leachate; and may also increase environmental pollution (Alimba *et al*., 2009).

Considering the geographical location of the state, there is rainfall almost every month of the year. The water table is high especially during the rainy seasons from April to October of each year. Due to the location of the landfills and or dumpsites, there has been chemical contamination of the underground water (Ikem *et al*., 2002), which is the main source of water for domestic and economic activities. Studies using different biomarkers have shown that chemicals present in leachates are deleterious to living cells at biologically hazardous concentrations (Wick and Dave, 2006; Koshy *et al*., 2007; Bhargav *et al*., 2008; Bakare, 2009; Bortolotte *et al*.,2009; Alimba *et al*., 2009). These chemicals can also bioaccumulate and biomagnify in a food chain (Cuadra *et al*., 2006). Fish has been identified as a major route for human exposure to xenobiotics in aquatic environment (WHO, 1990). The ease with which fish can be cultured in the laboratory and exposed to chemicals for *in vivo* genotoxicity studies may be significant in assessing health risk of higher vertebrates, especially human beings (Powers, 1989).

Information on the genotoxic potential of landfill leachate using fish as test organism is limited in spite of leachate polluting effects on the aquatic environments. Micronucleus (MN) test is one of the simplest short-term tests for biomonitoring the quality of aquatic environment (Al-Sabti and Metcalfe, 1995; Grisolia and Starling, 2001). MN in fish erythrocytes are quite distinct, easily scored and persist in the cytoplasm and their recognition is technically much easier (Ulupinar and Okumus, 2002). Genotoxic effects of chemicals using micronucleated erythrocytes of fish has been widely assessed both under field and laboratory conditions and has been considered suitable for cytogenetic studies in the monitoring of clastogenic and aneugenic chemicals (Odeigah and Osanyipeju, 1995; Grisolia and Corderio, 2000). Nuclear abnormalities (NAs) are produced when fishes are exposed to genotoxic and cytotoxic agents and are considered as indicators of genotoxic damage (Ayllon and Garcia-Vazuez, 2001; Cavas and Ergene-Gozukara, 2003). These NAs may be scored along with MN during genotoxicity studies.

With rapid increase in landfill leachate pollution of the aquatic environment, and subsequent endangering of fish species, it is therefore necessary to examine the potential toxic effect of leachate in fish. This study evaluates the genotoxic and cytotoxic potentials of leachate from Abule Egba landfill, Lagos, Nigeria using the MN test in peripheral erythrocytes of *Clarias gariepinus.*

Materials and Methods

Sampling site: Abule Egba landfill is located in Ojokoro Local Government Area on the Northwestern part of Lagos State (Latitude 6^{o} 64^{1}N and Longitude 3^{o} 30^{1}E), Nigeria. It covers about 10.50 hectares of land and receives an average of 25,625 tonnage of wastes per month [waste composition: vegetation/putrescible (68%), paper (10%), plastic (2%), nylon (5%), glass (4%), metal (3%), grit (4%) and textiles (4%) (Odunaiya, 2002)].

Leachate sampling, heavy metals and physico-chemical analysis: Raw leachate collected from 20 leachate wells at different points on the landfill, were filtered to remove debris and mixed in a precleaned 20 L plastic container. It was transported to the laboratory, designated as Abule Egba Leachate (AEL) and kept at 4^{o}C until use 72 hr later.

Standard physical and chemical parameters of the leachate including chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solid (TDS), chloride, sulphate, ammonia, alkalinity, phosphates, turbidity and nitrate were measured according to APHA (1998) and USEPA (1996). The concentrations of heavy metals: lead (Pb), iron (Fe), cadmium (Cd), copper (Cu), arsenate (As), manganese (Mn), chromium (Cr) and mercury (Hg) were determined using PerkinElmer® A3100 atomic absorption spectrophotometer.

Experimental procedure: The test organism is the juvenile of African catfish (*Clarias gariepinus*) obtained commercially from Ayode fish farms, Magodo, Lagos state, Nigeria. The fishes (average weight of 10.53 ± 0.47g and length of 8.94 ± 0.81cm) were acclimatized in the laboratory for 21 days in 100 litre transparent plastic tanks containing dechlorinated tap water. The water was aerated continuously at 25 ± 2^{o}C (using TECAX AP – 1500 air pump) and changed twice weekly. The fishes were fed with pellets (Nigeria Institute of Oceanography and Marine Research (NIOMR), Lagos feeds) *ad libitum*. Four concentrations 4.94, 7.42, 14.83 and 29.66% of the leachate sample (v/v; leachate:tap water) obtained after a range finding test (data not reported here) were utilised; Dechlorinated tap water was used as the control with 10 test animals in each concentration and the control. The leachate at the different concentrations and control group were changed twice weekly for 4 weeks. Natural photoperiodism of 12 hr light and dark cycle was maintained during the experiment.

At the end of the treatment, thin smear of peripheral blood, collected using heparinised syringe from the caudal vein of 5 fishes (randomly selected) per exposed and control groups, was immediately made on precleaned grease free slides. Three slides prepared per fish/ concentration was processed in accordance with Singh *et al*. (2005). Briefly, the smeared slides were allowed to air - dry over night at room temperature, fixed in absolute methanol for 20minutes and then stained with May-Grunwald and 5% Giemsa. The slides were analyzed at 1000x for MN and nuclear abnormalities. An MN was smaller than one-third of the main nucleus and did not touch the main nucleus. Cells having two nuclei with approximately equal sizes were scored as binucleated (Costa and Costa, 2007), while cells with rounded appearances and basophilic cytoplasm were scored as immatured erythrocytes (Lecklin *et al*., 2000). At least 1000 erythrocytes per fish were examined to determine the frequencies of micronucleated erythrocytes and nuclear abnormalities.

Statistical analysis: Data were analysed using SPSS 15® software. Statistical significance of the differences in mean ± SE values between treated and control groups, were determined with the Student's t-test at $p<0.001$ and $p<0.05$ level.

Results

Table1 shows the physico-chemical parameters and heavy metals analyzed in the leachate sample. AEL had a fouling odour and dark colour with a slightly alkaline pH value. The values of BOD, COD, TDS, chloride, sulphate, ammonia, phosphates, nitrate and the heavy metals analyzed (except Cu) were higher when compared with regulatory standards (Table 1).

The result on genotoxicity is presented in Table 2. The frequency of micronucleated erythrocytes increased in dose-dependent manner when compared with the control. The frequency of micronucleated erythrocytes increased in a dose-dependent manner when compares with the control and over four fold increase was found at the highest tested concentration of leachate sample (29.66%). This increase was statistically significant ($p<0.001$) at concentrations of 4.94 and 7.42% ($p < 0.05$) and 14.83 and 29.66%. The frequency of binucleated cells also increased significantly ($p<0.05$ and $p<0.001$) in a dose-dependent pattern compared with the control. Immature erythrocytes observed are mostly rubricytes stage. Which are smaller than mature erythrocytes and round to slightly oval in shape (Fig. 1, 2). The proportion of these immature erythrocytes in the circulating blood increased significantly ($p< 0.05$ and $p<0.001$) as the concentration of the leachate increased.

Discussion

Human beings are exposed during their life time to many chemicals in the environment. Landfill leachate pollution of the aquatic system may increase the level of human exposure to mixture of these chemicals. Many of these chemicals not only endanger the survival and physiology of the aquatic organisms

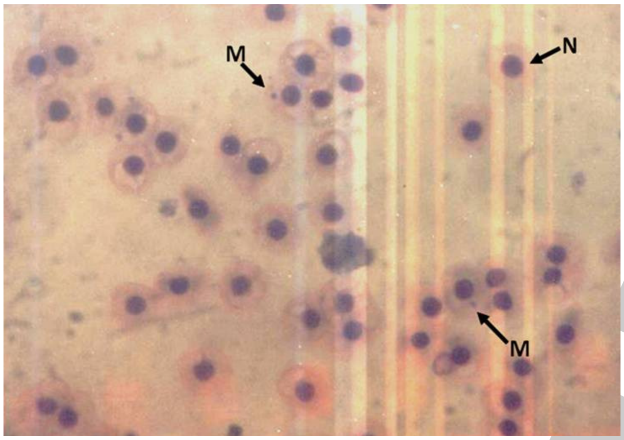

Fig. 1: Micronucleated erythrocytes (M) and normal erythrocyte (N) (1000x)

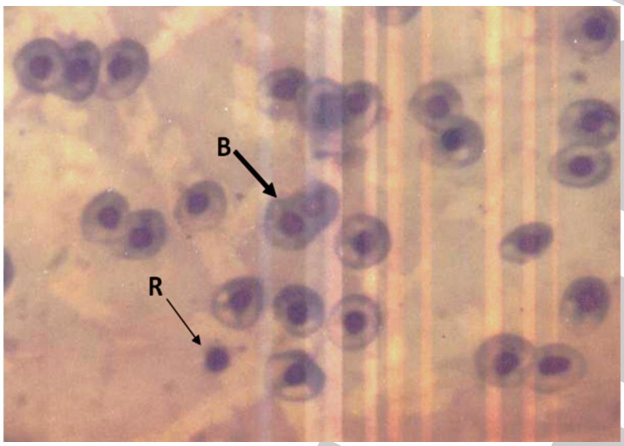

Fig. 2: Immature erythrocyte (R) and Binucleated erythrocyte (B) in *C. gariepinus* exposed landfill leachate (1000x)

but also induced genetic alterations which may enhance mutation and cancer formation (Russo *et al*., 2004). The consequences of these alterations may include reduced fitness, embryonic viability and genetic disorders in future generations (Kurelec, 1993). The results of the present study showed a high genotoxic potential of AEL in peripheral erythrocytes of *C. gariepinus*. The induction of MN was significantly higher in the treated groups than the control indicating that AEL contains clastogenic and or aneugenic chemicals capable of causing chromosomal damage and genome instability in *C. gariepinus*. Das and Nanda (1986) observed similar effects when they exposed Indian catfish (*Heteropneustes fossilis*) to mitomycin and paper mill effluent. Odeigah and Osanyipeju (1995) exposed *Clarias lazera* to ethyl methane sulfonate, textile mill and brewery effluents and observed concentration dependent increase in MN frequency in the erythrocytes. Pb, Cr, Ni and Hg analyzed in AEL have been reported to be mutagenic or clastogenic and have induced MN formation and DNA damage in peripheral blood lymphocytes of human and mice respectively (Saleha *et al*., 2001; Minozzo *et al*., 2004; Danadevi *et al*., 2004). Al-Sabti and Hardig (1990) also reported increased MN frequencies in erythrocytes

Table - 1: Physical and chemical characteristics of Abule – Egba Raw leachate

Parameters+	AEL	USEPA*	NESREA#
pH	7.9	6.5 – 8.5	6.0 – 9.0
TDS[a]	3591.32	500	500
Alkalinity	3080	20	150
BOD[b]	121.83	-	50
COD[c]	268.54	410	90
Turbidity	45	-	5.0
Ammonia	88.96	0.02	1.0
Nitrate	78.69	10	10
Phosphate	10.72	-	2.0
Chloride	1076.36	250	250
Sulphate	726	250	250
Cu	0.21	1.0	0.5
Fe	11.36	0.3	-
Pb	10.11	0.015	0.05
Cd	12.78	0.05	0.2
Mn	2.61	-	0.2
Cr	3.61	0.01	0.05
As	4.38	-	-

+ All values are in mg L^{-1}, except pH without unit and turbidity in (NTU)
*United State Environmental Protection Agency (1996) (www.epa.gov/safewater/mcl.html)
National Environmental Standards and Regulations Enforcement Agency (2009) (Nigeria) maximum permissible limits for effluent from wastewater.
[a]TDS- Total dissolved solids
[b]BOD-Biochemical oxygen demand
[c]COD-Chemical oxygen demand

of fish, *Carassius auratus gibelio*, treated with Cr^{3+}. The report of Deguchi *et al*. (2007) is similar to our observation except that they had difficulty in detecting MN in peripheral erythrocytes, but in gill cells of gold fish (*C. auratus*) exposed to raw landfill leachate. The slight difference from our results may be due to physiological differences of fish species (Haux *et al*., 1986) and duration of exposure of fish to the leachate samples.

The simultaneous expression of morphological nuclear abnormalities and MN may constitute a better method of assessing cytogenetic damage of chemicals to aquatic forms. The increase in binucleated cells in this study further highlight the cytogenotoxic effects of landfill leachates. It indicates that landfill leachate contains chemicals that are capable of blocking cytokinesis of a normal dividing cell during M phase of the cell cycle leading to the formation of a cell with two nuclei (Fenech *et al*., 2003). Binuclei formation in cells may lead to genetic imbalance and carcinogenesis (Rodilla, 1993). Similar observations were reported by Cavas and Ergene-Gozukara (2003) and Ali *et al*. (2008) when they exposed *Orechromis niloticus, O. aureus, Tilapia zilli* and *C. gariepinus* to a textile mill effluent contaminated water and cyclophosphamide.

The concentration dependent increase in the frequency of immature erythrocytes indicates that AEL contains cytotoxic

Table - 2: Frequencies of micronuclei (MN), binuclei (BN) and immature erythrocytes in *C gariepinus* exposed to Abule Egba Landfill leachate.

Concentration of leachate (%)	Total no. of cells scored	Cells with MN (Mean ± SE)	Cells with BN (Mean ± SE)	Immature cells (mean ± SE)
Tap water	11,768	0.37 ± 0.03	1.02 ± 0.12	0.93 ± 0.09
4.94	10,656	1.78 ± 0.46*	2.21 ± 0.61*	1.99± 0.18
7.42	11,081	2.41 ± 0.32*	3.81 ± 0.32*	3.11 ± 0.44*
14.83	10,002	2.89 ± 0.83**	5.02 ± 0.91**	3.89 ± 0.71**
29.66	96,789	4.08 ± 0.68**	7.18 ± 0.11**	4.32 ± 0.32**

*p<0.05 , **p<0.001

chemicals. Immature erythrocytes, especially the rubricyte stages observed herein are rarely seen in peripheral blood films of lower vertebrates including birds. Their presence may indicate a marked erythropoietic response or erythrocyte dyscrasia such as erythroblastosis (Campbell, 2004). Similar observation was found in circulating blood of *Tilapia* after short term exposure to Aluminum (Alwan *et al*., 2009). Birds suffering from heavy metal toxicosis, especially lead poisoning, often reveal an inappropriate release of immature erythrocytes (Campbell, 2004). The increase in immature erythrocytes may be due to the effects of the heavy metals and other chemicals not characterized in this study which caused the breakdown of mature erythrocytes (as evidenced by decrease in the number of cells counted compared to the control, Table 2) hence, a compensatory erythropoiesis occurred.

Some chemicals including heavy metals can bind to phosphate and base residues of DNA, to alter its primary and secondary structures (Bridgewater *et al*., 1994; Buttke and Sandstrom, 1994). They are also capable of interfering with protein structure and function to cause DNA damage (Resit *et al*., 1998). Also free radical generation and oxidative damage by these chemicals may be responsible for MN formation in fish erythrocytes. This assumption may be supported by the study of Li *et al*. (2006), wherein they showed that MN induction by landfill leachate in the bone marrow cells of mouse may be due to free radical generation and oxidative damage caused by chemicals present in the leachate. Fish metabolize many carcinogens in a manner analogous to mammalian system (Stegeman and Lech, 1991), and also respond to chemicals that induced oxidative damage in hepatocytes and hepatic cytochrome P-450 mono-oxygenases in similar manner to mammalian species (Washburn and Di Giulio, 1989; Goksoyr *et al*., 1991).

The values of BOD and COD in AEL indicate it may contain high concentration of organic compounds which are highly toxic to aquatic systems (Odunaiya, 2002). Paxeus (2000) detected over 200 organic compounds in a municipal landfill leachate and reported that 35 among them are potentially harmful to the environment and human health. The discharge of leachate with high organic compounds into aquatic environment may cause an increase in growth of microorganisms (Oshode *et al*., 2008) by acting as substrates and may lead to oxygen depletion of the water environment. Also large quantities of suspended and dissolved solids may increase water turbidity reducing the light available to photosynthetic organisms. Nitrate detected in AEL exceeded safety limits (Table1); its high concentration in water may result in the formation of nitrosamines which are associated with cancer of the digestive tract, urinary tract and non-Hodgkin lymphoma (Guilis *et al*., 2002).

Fishes are well known sentinel organisms; they are capable of indicating the potential for human exposure to genotoxic chemicals (Miracle and Ankley, 2005). The results of this study showed that xenobiotics present in Abule Egba landfill leachate induced MN and BN in the erythrocytes and also increase the concentration of immature erythrocytes in *C. gariepinus* in a concentration dependent manner. This shows the cytogenetoxic effects of landfill leachates on aquatic forms.

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