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# Industrial Effluent Induced Chromosomal Aberration in Catfish from Ogun River, Lagos, Nigeria

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**Abstract:** The aim of the study is to evaluate the cytotoxic effect of effluents at inducing chromosomal aberrations, using this as a biomarker tool in wild *Clarias pachynema* for assessing and monitoring pollution of the aquatic environment. A total of 60 live fish (30 each downstream and upstream) were obtained and subjected to chromosomal analysis. Chromosomal aberration in the fish samples from the downstream sector was recorded at a rate of 30%, while there were no aberrations in the samples collected upstream the effluent discharge point. Water sample analysis revealed a high concentration of Ammonia and Nitrates above permissible standards of Federal Environmental Protection Agency (FEPA) guidelines. Heavy metal analysis also revealed the presence of Cr (0.05), Cu (0.01), Pb (0.05), Zn (5.0) and Fe (0.3) above permissible standards from the downstream section of the river. This study shows clearly that the ever increasing discharge of effluents from the industry could increase chromosomal damage in the aquatic components.

**Key words:** Chromosomal aberration, environmental pollution, geno-toxicology, fresh water fish, biomarkers

## INTRODUCTION

Human activities resulting from industrialization, technology and farminginvolve the use of chemicals which find their way into water bodies through run offs and erosion and contribute to disturbance of the aquatic ecosystem (Wang, 2002; Dautremepuits *et al*., 2004). The toxic effects of chemical compounds in the aquatic body are diverse depending on the type of compound which could either act alone or synergistically, especially in mixtures of chemicals (UKWIR, 2002). Thus, depending on the quantities and toxicological potentials of dumped or spilled chemicals and the character of the receiving environment (flow rates. temperature, sediments and biota in a stream) impacts (often acute death losses) will vary. Heavy metals are not biodegradable and can accumulate in the environment, thus, they may become bioavailable to aquatic organisms and the humans who consume aquatic products. Heavy metals can accumulate in the tissues of aquatic animals and as such tissue concentrations of heavy metals can be of public health concern to both animals and humans (Kalay *et al*., 1999; Ashraf, 2005).

Chronic exposures to heavy metals have debilitating effects which sometimes go unnoticed. It could manifest as changes in population structure, altered reproduction patterns and growth retardation which are important considerations for sustainable intra- and interspecies diversity (Kumar and Mathur, 1991).

The growth in numbers and volumes of chemicals, both man-made and natural that have been released into the environment over the past fifty years has been immense. In 1942, only six hundred thousand (600,000) chemicals were known. This number has now increased to almost eleven million (11,000,000) (Sveltana *et al*., 2004) and most of these compounds are neurotoxic, nephrotoxic, hepatotoxic, carcinogenic and non-biodegradable.

The most important ecological aspect of chemical carcinogens in the environment may be the genotoxic potential of the carcinogens. It is essential to know, therefore, what effects, if any, water-borne pollutants have on the genetic material of aquatic organisms, particularly fish (Dixon *et al*., 1999). The alarming increase in industrialization and urbanization over the past five decades both in developed and developing nations have further heightened the ecological and toxicological problems that result from release of toxic chemicals into the environment (Orloff and Falk, 2003). These releases have triggered, internationally, an increasing concern about the impacts of these chemicals on the health of the environment. To date, there is no single instrument that can measure the full potential of toxic impacts of a single chemical let alone the complex mixtures of chemicals as

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occur in the ambient environment. Living organisms, however, serve as sensitive indicators of toxicity, provided that they are astutely monitored (True and Hayward, 1990).

Toxic pollutants often cause characteristic responses in the affected organism, commonly known as 'toxicological endpoints' or 'biomarkers'. A biomarker, as defined by Depledge *et al*. (1993), as "a biochemical, genetic, cellular, physiological or behavioural variation that can be measured in tissue or body fluid samples or at the level of the whole organism (either individuals or populations), that provides evidence of exposure and/or effects of one or more chemical pollutants and/or radiation". Biomarkers are powerful tools for detecting the impact of exposure to sub-lethal concentrations of a given substance or complex chemical mixtures, enabling the evaluation of less obvious effects on organisms.

In aquatic ecotoxicology, the use of biomarkers has traditionally been applied to the exposure of sentinel organisms or *in vitro* test systems to pollutants in aqueous solutions or suspensions. These approaches are designed to provide guidelines for legislation which targets the impacts of human activities on marine and fresh water environments. Measurements in selected species of aquatic organisms of biological responses (biomarkers) related to exposure effect and susceptibility to pollutants is a useful approach that links the bio-availability of pollutants and intrinsic toxicity in target organs (Van der Oost *et al*., 2003).

There has been in recent years, relative improvement of water quality in many areas, but water sediments may still serve as sources of many persistent chemicals (Harris *et al*., 1996) thus shifting the focus of ecotoxicological studies towards sediments and the potential deleterious effects persistent pollutants have on benthic ecosystems (Anderson *et al*., 1996). Many studies have reported the mutagenic, clastogenic and carcinogenic effects of metals in mammals (Buchet *et al*., 1980; Bates *et al*., 1992; Fowler *et al*., 1994; Elinder and Jarup, 1996; Godet *et al*., 1996; Galaris and Evangelou, 2002; Alimba *et al*., 2006; Bakare *et al*., 2007). Heavy metals like chromium, copper and iron were reported to bioaccumulate in *Clarias gariepinus* and zinc and copper in *Oreochromis niloticus* (Kotze *et al*., 1999). More recently, Wright *et al*., 2010 reported that environmental metal exposures show evidence of changes in epigenetic marks which points to a possible link between heritable changes in gene expression and susceptibility to diseases. The mechanism of induction of chromosomal aberrations in fish though, has not been well elucidated.

Numerous fish species are important sources of protein and other nutrients in the diets of man, it is imperative therefore, to know whether mutagenicity in fish can serve as an early warning of potential risks both for the fish and the human or non-human consumer of the contaminated fish or whether the fish can act as sources of transmission of mutagenic chemicals to consumers of their tissues. The selection of fish as a model in ecotoxicologic studies could be valuable as fish serves as a very sensitive bio-indicator of aquatic contamination in tropical regions (Mdegela *et al*., 2006). Fish play important roles in the trophic web, as they bioaccumulate or biomagnify certain environmental pollutants and biotransform some of them to less or more toxic metabolites (Goksoyr, 1991).

While it is essential that thorough mutagenic screening be performed in mammals, it is equally important to evaluate genetic damage that might occur in species man depend on for food and recreation (Kligerman *et al*., 1975). This research work, deals with the cytologic effects of effluents discharged into River Ogun at IsheriOlofin using *Clarias* species from the water as *in situ* sentinels. After the damming of the river for Lagos state water supply scheme at Akute, the River runs through IsheriOlofin before bifurcation into two tributaries. One tributary discharges into the Lagos lagoon at Oworonsoki area in Lagos while the other does the same at Ikorodu. Apart from intense dredging for sand, other major activity at this end of the river is that of abattoir sited proximate to the river and receives effluents being channeled from the slaughter slab of the abattoir on daily basis. The abattoir activities include killing of the cows and other ruminant animals, carcass dressing, as well as evacuation of the gut contents of the killed animals, which chiefly constitute the effluents being discharged into the river. This abattoir location end of the river falls in the boundary area of Lagos and Ogun State end of Lagos-Ibadan expressway. Physico-chemical analysis of water samples was carried out from upstream and downstream of the River and metals analyzed were Copper (Cu), Cadmium (Cd), Cromium (Cr), Iron (Fe), Lead (Pb) and Zinc (Zn). The pH, nitrates and ammonia levels were also determined from upstream and downstream of the River.

## MATERIALS AND METHODS

**Sample sites:** River Ogun is one of the major rivers in Nigeria. It has an approximate surface area of 2,237,000 ha. Its source lies within 3°28'E, 8°41'N in Oyo state and enters Lagos lagoon at a point on longitude 3°25' and latitude 6°3' (Adebisi, 1981). Ajao *et al*. (1996) named it as one of the major and important rivers draining more than 103,626 km$^2$ of the country into the Lagos lagoon.

**Animal model:** A total of 60 fish specimens from IsheriOlofin were used for this study, 30 samples each, collected from the upstream and the downstream sections of the River from July-September 2008. These were brought to the Aquaria section of the Federal College of Animal Health and Production Technology, Institute of Agriculture, Research and Training, Ibadan and kept in aerated tanks for 24 h to acclimatize.

**Preparation of chromosomes:** Preparation of chromosomes was carried out according to Adeogun (2007) (unpublished) *in vivo* preparation of fish chromosomes, modified after the method prescribed by Geber and Schweizer (1988). Colchicine® was injected intraperitoneally and the fish left in water for 4h. After which the fish were stunned by a sharp blow to the head and subsequently decapitated and the kidneys dissected out. Colchicine can cause the mitotic arrest of dividing cells (both plant and animal cells) at metaphase by interfering with microtubule organization, in particular, those of the mitotic spindle (Andreu and Timasheff, 1982).

Briefly, the kidneys were teased in 2 mL, 0.56%, 0.75M KCl in a glass Petri dish. Cell suspension was then poured into 15 mL centrifuge tubes and 5 mL KCL were added and left for 30 min at room temperature. Cell suspensions were then divided into five 1.5 mL Eppendorf tubes and immediately slowly overlaid with 0.2 mL chilled Carnoy's fixative (methanol: acetic acid, ratio 3:1) and each sample suspension gently mixed. Centrifugation of cell suspensions was done at 2000 rpm for 10 min at room temperature and the supernatants removed. Sediments were overlaid with 1.2 mL of chilled Carnoy's fixative and kept at 4° for 30 min to ensure thorough fixation. Centrifugation and supernatant removal was repeated three times until a clear transparent suspension was obtained.

**Staining technique:** Each time, a small quantity of cell suspension was obtained and dropped onto a grease free, pre-cleaned glass slide from a height of 1-1.5 feet for good spread. The slides were allowed to air dry. After 24 h, each slide was stained with 5% Giemsa for 20 min after which it was washed in distilled water and air dried again. Permanent preparations were made by mounting in synthetic neutral mountant (Canada balsam). Metaphase spreads were observed under the microscope and photomicrographs were taken at X100 magnification. Samples slides were examined for chromosomal aberrations (both numerical and structural). Eight slides were prepared per fish and a total number of 200 spreads were each examined, from the upstream and downstream sectors.

Water samples collected upstream and downstream in 1 L bottles were transported in ice packs were sent to the water chemistry Laboratory of the Institute of Agriculture Research and Training for analysis.

## RESULTS

Results of the physico-chemical analysis of the water samples downstream were compared with FEPA and United States Environmental Protection Agency (USEPA) standards (Table 1). The pH of the water sample was within the acceptable limits of FEPA and USEPA recommendation. However, the nitrate and ammonia concentrations of 24.8 and 0.45 mg $L^{-1}$ obtained downstream were higher than the acceptable limits of 20 and 0.01 and 10 and 0.02 mg $L^{-1}$ for FEPA and USEPA, respectively. The downstream water also appeared cloudy compared to the upstream sample. The water samples also contained concentrations of copper (Cu), chromium (Cr), iron (Fe), lead (Pb) and zinc (Zn) that were above the standards set by the FEPA, USEPA and WHO (Table 2). By contrast, the levels of cadmium fell below the detection limits. The fish samples used for the study were identified as *C. pachynema* (Teugels, 1984) using morphologic parameters. Chromosomal aberrations were detected and scored. Analysis of chromosome spreads reveals that breaks, rings, acentric and dicentric (Fig. 1, 2) chromosomes, were found in approximately 30% of the metaphase of each of the fish examined (x = 29.86+5.97) for the downstream samples (Table 3). No aberrations were found in the samples collected upstream.

Table 1: Physico-chemical characteristics of Ogun River at Isheri Olofin, Lagos Nigeria

| Parameters* | Ogun river (downstream) | Ogun river (upstream) | FEPA[a] | USEPA[b] |
|---|---|---|---|---|
| pH | 7.50 | 7.10 | 6-9 | 6.5-8.5 |
| Nitrate | 24.80 | 8.00 | 20 | 10 |
| Ammonia | 0.45 | 0.15 | 0.01 | 0.02 |

*All values are in mg $L^{-1}$ except pH, [a]Federal environmental pollution agency FEPA (1991) Guidelines for uniform effluent limits in Nigeria for all categories of industries, [b]United states environmental protection agency (www.ces.purdue.edu/extmedia/wq/wq-5.html)

Table 2: Heavy metals detected in the water sample collected from the Ogun River at the IsheriOlofin effluent discharge site

| Parameters* | Ogun river (downstream) | Ogun river (upstream) | FEPA[a] | USEPA[b] | WHO[c] |
|---|---|---|---|---|---|
| Cu | 4.60 | 3.00 | 0.01 | 1.000 | 2.000 |
| Cd | 0.00 | 0.00 | 0.01 | 0.005 | 0.003 |
| Cr | 0.35 | 0.17 | 0.05 | 0.100 | 0.050 |
| Fe | 3.62 | 1.00 | 0.30 | 0.300 | - |
| Pb | 0.14 | 0.10 | 0.05 | 0.015 | 0.010 |
| Zn | 35.60 | 7.40 | 5.00 | 5.000 | - |

*All values are in mg $L^{-1}$, ND: Not detected, [a]Federal environmental pollution agency (2001) Permissible limits for drinking water, [b]USEPA; United states environmental protection agency www.epa.gov/safewater/mcl.html, [c]WHO; World Health Organization guidelines for drinking water quality

Table 3: Analysis of chromosomal aberrations in *Clarias pachyne*ma from Ogun River at Isheri Olofin

| Fish code letter | No. of metaphases examined | No. of metaphases with at least one aberration | Total No. of aberrations | % of Metaphases with at least one aberration | Aberrations per metaphase |
|---|---|---|---|---|---|
| OG[1] | 193 | 45 | 66 | 23.32 | 0.3420 |
| OG[2] | 166 | 52 | 76 | 31.33 | 0.4578 |
| OG[3] | 140 | 49 | 83 | 35.00 | 0.5929 |
| Total | 499 | 146 | 225 | 29.88±5.97 | 0.4642±0.126 |

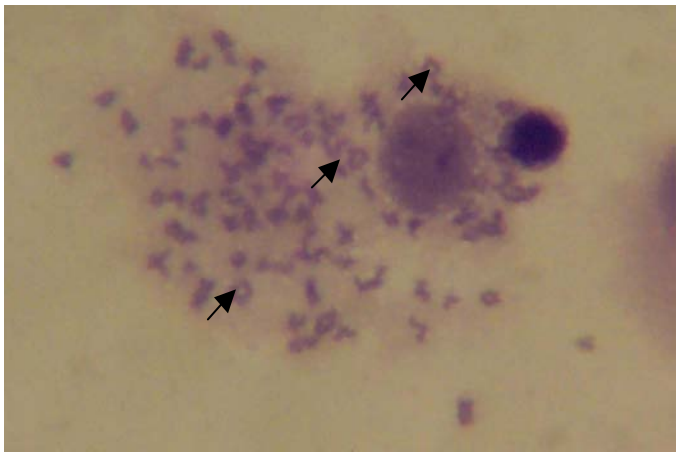

Fig. 1: Ring form chromosome (arrow heads) observed in the metaphase spread of *Clarias pachynema* from Ogun River at Isheri Olofin

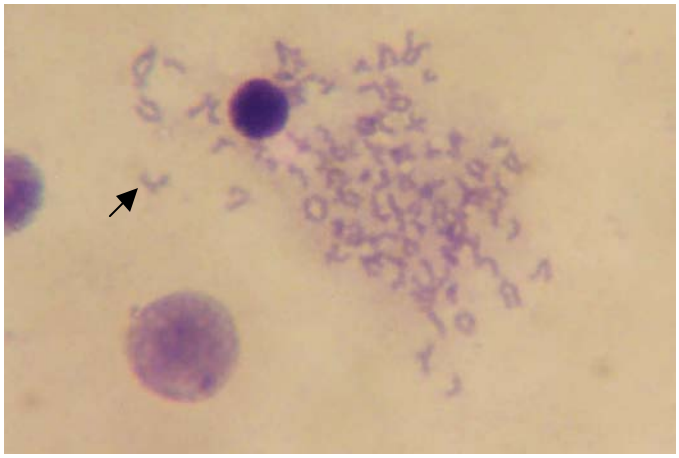

Fig. 2: Dicentric chromosomes observed in the metaphase spread of *Clarias pachynema* from Ogun River at Isheri Olofin

## DISCUSSION

Most assessments of the toxic potential of aquatic contaminants to fish carried out to date in Nigeria have involved the analysis of various organs. However, to our knowledge, none has been published on chromosomal aberrations in fish as it relates to environmental toxicity. Chromosomal damage was assessed in *Clarias pachynema* found in Ogun River at Isheri Olofin. The findings showed that the river is polluted downstream and this may have contributed to induced chromosomal aberrations in *Clarias pachynema*. The

potentially dangerous amount of the toxic water samples is likely due to its constituent chemicals (abattoir effluent is discharged on a continuous basis into the river). This research reports an increased incidence of chromosomal aberrations in fishes downstream a polluted waterway compared to their counterparts upstream. The dichotomy of toxicity can be observed in an upstream and downstream waterway (Huff *et al*., 1991), reported winter flounder from polluted waters were found to have visible lesions on the liver, while the winter flounder outside the estuary had no lesions. The major sources of heavy metal contamination in aquatic environments are industrial effluents, sewage disposal, soil leaching and rainfall. In this study, heavy metals such as Cu, Cr, Fe, Pb and Zn were detected in higher quantities in water sample downstream. Aquatic organisms are known to accumulate significant amount of metals from their environment (Van de Mewer *et al*., 1990).

In this regard, it would be noteworthy to state that an earlier study reported the accumulation of Cd, Zn, Pb and Cu in high concentrations in the liver, kidney, heart and gills of *Clarias gariepinus* from the same River, which led to induction of lipid peroxidation and alteration in the antioxidant enzymes in the organs of the fish (Farombi *et al*., 2007). Research studies show that polluting substances such as heavy metals could act on organisms directly and/or form free oxygen radicals, which can initiate degenerative processes and cause genotoxic effects (Di Giulio *et al*., 1989; Halliwell and Gutteridge, 2000; Bakare *et al*., 2007).

A total of 30% chromosomal aberration was detected in fish downstream with elevated heavy metals concentrations in the water. Barsiene (1995), reported that fish are good pollutant bioaccumulators and the consequence of pollutants to which they are exposed are displayed first at the biochemical and then at molecular levels in tissues evidenced by genetical changes which become cytologically visible. He also reported that heavy metals (namely Fe, Mn, Cu, Zn, Ni, Cd, Pb, Cr) and aromatic hydrocarbons (naphthalene, anthracene, phenanthrene, fluoranthene, pyrene, triphenylene, chrizene, perylene, 3-4 benzoapyrene) caused an enhanced frequency of chromosomal aberrations in molluscs.

A study conducted with the chromosomes of central mud minnows showed that the fish specimens could be used as valuable model organisms for studying the clastogenic effects of waterborne chemicals (Ulupinar and Okumus, 2002). An average rate of 30% chromosomal damage observed in fish collected downstream in this study is similar to that observed by Kligerman *et al*. (1975), who detected chromosome aberrations in *Umbra limi* (mud minnow fish) after exposing them to X-radiation *in vitro*. This provides strong evidence that the constituents of the water samples may be highly clastogenic, inducing chromosomal damage at the same rate as those of X-rays, which were reported to induce chromosomal aberrations through the formation of free radicals.

The presence of nitrate and ammonia in the downstream water sample at concentrations above acceptable was likewise detected. These compounds have been associated with cancer of the digestive tract. In the gastrointestinal tract, nitrite can interact with amines formed from the breakdown of proteins to produce nitrosamines, a class of chemicals that is highly carcinogenic in experimental animals (Reif, 1981). These studies clearly reveals the genotoxic potential of waste water effluent since, fishes can respond to mutagens at low concentration of toxicant in a manner similar to higher vertebrates (Goksoyr, 1991).

## CONCLUSION

The measurement of chromosomal aberrations appears to offer an acceptable parameter for monitoring mutagenic substances in water and that fish could be a useful cytogenetic model for monitoring pollution in the aquatic environment. In order to standardise this procedure, a larger suite of chemicals in water should be analysed, screening involving a larger number of fish carried out and the cell cycle kinetics carried out for the chromosomal aberrations to become more purposeful. It should be noted however, that the heavy metals and compounds included in our study do not represent all or even the majority of chemical species in the water samples. Hence, the cause of genotoxicity in the fish samples could have been due to one or a combination of known and unknown components.

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