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Micronucleus Assay-An Effective Tool to Assess the Genotoxicity with Special Reference to Fishes

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ABSTRACT

Due to various anthropogenic activities and discharge of agricultural and industrial waste into the aquatic environment the aquatic ecosystem is facing a great problem of bioaccumulation of genotoxic contaminants like heavy metals and other polycyclic hydro-carbon. These genotoxins accumulate in the various tissues of the organism causing change in chromosomal structure and aberration at gene level. Fish accumulates these genotoxins and acts as an bioindicator of pollution and thus helps in biomonitoring of the aquatic environment. To assess the genotoxicity of these contaminants a number of assays and techniques are used like DNAs' OH end labeling, single cell Gel electrophoresis or Comet Assays and Chromosomal aberrations test (CAT). Among them is MN assay or MN Test (Micronucleus test) is a very simple and most widely used tool to assess genotoxicity.

Key words: *Genotoxicity, Bioaccumulation, MN assay, Fish*

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INTRODUCTION

Today we are living in an era full of environmental stress in terms of pollution related toxicity and bioaccumulation of genotoxic Contaminants like heavy metals and polycyclic hydrocarbons in the aquatic environment. The contamination of water is due to various anthropogenic activities and discharge of agricultural and industrial waste into the aquatic environment. (Bhuvaneshwari *et al.,* 2012). These water contaminants may cause ecological imbalance in the aquatic environment causing loss of aquatic biodiversity (Ashraj,2005; Vosyliene and Jankaite, 2006). So, the aquatic contamination by these chemicals is of great concern (Dirilinger, 2001; Vutukuru, 2005; Yousufzai and Shakoori, 2006; Fatoki *et al*., 2012; Okoro *et al*., 2013; Okoro *et al*.2014a; Okoro *et al*., 2014b; Hussein Kehinde *et al*., 2016) .These genotoxins are the pollutants with mutagens and carcinogens that directly affect the genome of an organism. These may include a number of chemical compounds like heavy metals (Matsumoto *et al*., 2005; Matsumoto, 2003; Pruski and Dixon, 2002) and PAH (Polycyclic aromatic hydrocarbons) (*IARC,* 1983; Santodonato *et al.,* 1981; Black *et al.,* 1983; Germain *et al.,* 1993). These genotoxins can directly affect the germ cells and as a result genetic changes are transferred into the next generations (Hartwell *et al.,* 2000).

Apart from being a great source of food for the world population, (Blasco *et al.,* 1998; Agah *et al.,* 2009) fish has been the best model for the research. Fishes act as a magnificent bio indicator of aquatic pollution and genotoxicity as they accumulate these genotoxins in various body tissues (Grisolia and Cordirio, 2000). As a bioindicator, fishes indicate the introduction of chemical toxins in the aquatic environment (*Bailey, et al.,* 1992). Because fishes are having nucleated erythrocytes, the MN test in erythrocytes has been widely used in different fish species to assess the aquatic pollutants showing mutagenic effects (Saotome and Hayashi, 2003; Pantaeao *et al.,* 2006). They respond to mutagens at low concentrations (Gksoyr *et al.,* 1991). Moreover, they are more sensitive for the induction of DNA damage in comparison to mammals. (Raisuddin and Jha, 2004).

To assess the aquatic genotoxicity of these contaminants, MN Assay or MN Test (Micronucleus Test) is the most widely used tool due to its simple approach. The MN Assay is sensitive, fast and biomarker of mutagenic exposure in the environment (Carlos *et al.,* 2009). It is an authentic tool for testing chemicals responsible for the induction of numerical and structural chromosomal damage. It provides important information about the ability of a chemical to interfere with chromosomal structure and function (ntp.nich.nih.gov. 2016) .A proper knowledge of MN assay is very useful to assist laboratories in assessing the water quality by developing the fish genotoxicity assays (Al Sabti *et al.,* 1995).

Therefore, in the present article, an attempt has been made to gather the research data related to the use of Micronucleus Assay with special reference to fishes.

What is a micronucleus?

A micronucleus may be defined as a small micronucleus that is formed as a consequence of having aberrated chromosomal fragments or chromosomes or whole chromosomes that are not incorporated into the nucleus after cell division. A micronucleus may be defined as a small micronucleus after the main nucleus that is formed as a consequence of various clastogenic and aneugenic effects of a number of genotoxic agents. Being induced by these genotoxins, these micronuclei contain damaged chromosomal fragments. During the mechanism of cell division these small fragments are not incorporated into the nucleus and become what we define as micronucleus.It is important to know that the formation of micronuclei can only be observed in cells undergoing all

division (Fenech *et al.* ,2011).Chemically induced micronuclei was first reported in Ehrlich-ascites Tumor Cells treated with colchicines. (Fenech *et al.,* 2011).Prevalence of micronucleus cells is closely associated with the interference mitosis and chromosomal breakage. (Tolbert *et al*., 1991)

The protocol and the methods for M N Assay have been used in most of the studies according to Tolbert *et al.* (1992) and Al-Sabti et al. (1995).

Micronucleus test in Fresh water fishes

Micronucleus test is an authentic tool to study the in situ assessment of the biomarkers for biomonitoring of freshwater ecosystems. Rodrigue *et al.(* 2003) studied the induction of micronuclei in three freshwater fish species -brown trout *Salmo trutta, Anguilla anguilla* and *Phoxinus phoxinus* and demonstrated the sensitivity towards the polluted sites and showed that trout are more sensitive to micronucleus test. Their in situ survey revealed that these trouts acted as in situ pollution biomarkers by measuring micronucleus indices of their renal erythrocytes.

The in vitro micronucleus assay has been used to characterize the origin of micronuclei induced by cyclophosphamide (Fagr *et al.* 2008) Genotoxicity of PFF (Profenofos) in fish has been investigated in *Channa Punctata* as a model fish by using the MN Test .The study shows that PFF is toxic to aquatic organism and micronucleus test is an authentic tool to determine the potential genotoxicity and mutagenicity of xenobiotic compounds. The MN test is an important tool for monitoring toxicity (Pandey, Nagpure and Trivedi, 2014).

According to Carlos *et al.* (2009), MN Assay is sensitive, fast and biomarker of mutagenic exposure in the environment. They studied the micronuclei and other nuclear abnormalities in erythrocytes of *Colossoma macropomum* exposed to methyl mercury.

The micronucleus test is very useful in Cytogenetic of various categories of genotoxic substances including effluents from petroleum refinery plants. The analysis by MN test showed potential of these genotoxic effluents. (Tolga Cavas *et al.,* 2005).

Studies showed that a cytogenetic analysis by MN test in fish erythrocytes acts as a biomarker for marine environmental contamination. The evolution of MN frequency baseline was done in a number of species (*Scophthalmus maximus*) and determination of genotoxic potential of a number chemical contaminants was done (dialkyl phthalate; bisphenol A, Tetrabromodiphenyl ether) exposed in aquarium under controlled conditions .Micronucleus test is proved to be very authentic in the genotoxic analysis in natural water as well as in experimental condition(Claudia *et al*.,2006) . It has been concluded that the micronucleus test is a sensitive model to evaluate genotoxic compounds in fish under experimental conditions.

Hussein Kehindi *et al.,* (2016) used the micronucleus assay to evaluate the extent of genetic damage caused by accumulation of heavy metal in various tissue of species *Tilapia zilli, Oreochromis niloticus* and *Sarotherodon galilaeus* from a polluted river, Nigeria.

A study using the micronucleus test was carried out for the analysis of environmental quality by Leonardo and Elisabete (2010) in two estuarine systems under different degrees of contamination. The formation of micronuclei was observed only in one of the estuaries (90,000 cells observed) while other, though minor in number, did not show formation (48,000 cells observed) confirming the low degree contamination in the latter. Similar study was also carried out by

AZevedo *et al.* (2009). Azevedo (2008) and Oliveria (2007) observed low frequencies of micronucleus by using the MN assay in one of the estuary

Micronucleus test was carried out to test the genotoxicity in barbell (*Barbus Barbus*) from the natural population in the Danube river (Boettcher *et al.,* 2010).

The Genotoxic effect of Cr (III) was studied using fish MN analysis in peripheral blood Erythrocytes from Pimephales Promelas, the fathead minnow (De-Lemos *et al.*, 2001). A significant micronucleated erythrocytes (MNE) induction was observed in fish exposed to Cr (VI) at various periods. These reports may be helpful for further assay quality control (M I Mir *et al.,* 2014).

Nuclear abnormalities using MN test were reported in *Oreochromis niloticus* exposed to petroleum refinany or chromium processing plant effluents .The study showed genotoxic potential of both the effluents in the fish (Canvas and Ergen Gozukara, 2005; Mir et al., 2014). Similar results were also reported in *Cyprinus Carpio* (Zhu *et al.,* 2004).

During using the MN test with *Synodontis clarias* and *Tilapia Nilotica* from freshwater of the Anambra River, *Obiakor et al.,* (2010a) reported that micronuclei can easily be observed in erythrocytes and thus can be used as a measure of chromosomal aberration. They validated the micronuclei rates of these species as an index of cytogenetic damage and thus proved the NM test as an authentic tool for monitoring of these genotoxins. Obiakor *et al*.(2010C) carried out the Karyomorphological analysis and micronucleus assay in *Clarias gariepinus* exposed to water to River Oyi. The water of River Oyi have been under high pollution stress (Obiakar *et al.,* 2010C) Ahmed *et al.,* (2002) in their study revealed that the frequency of micronuclei by using MN test increases with increased time of exposure of pentachlorophenol. Morphological variations of erythrocytes by computer image analysis showed a 1:5 ratio of micronuclei and main nucleus with a reduced cell volume.

Fagr *et al.*, (2008) reported that micronucleus assay in fish Genome is a sensitive test for mentioning the pollution. They used three species of *Tilapia* (*Oreochromis niloticus, Oreochromis aureus* and *Tilapia cilli)*) and Clarias *gariepinus* from four locations representing different levels of contaminants in Egypt (Ali and El-Shehawi*,* 2007). The study revealed the few fish species showed different degrees of sensitivity to maintain genetic damage. This can be shown by variations in average of the micronuclei formed in cells among species at different locations. These locations showed differential environmental stress. (*Ali* and *El-Shehawi* 2007).

According to Fagr *et al.,* (2008) the peripheral blood of *Clarias gariepinus* is very sensitive in the formation of micronuclei corresponding with that of environmental stress. A comparative study between the micronueli frequencies of kidney and gill erythrocytes was carried out by Palhares and Grisolia (2002) in *Tilapia* to assess the Genotoxicity of chemical contaminants.

Obiakor (2010) and Obiakor *et al*. (2010a) studied the genotoxic status of the Anambra River by using MN assay with special reference to two dominant species of the river and reported possible cause of congenital disease outbreak among the fishes. According to Fenech et al. (2003) while using MN Test, the fish species showed variation in micronucleus frequencies in peripheral and kidney blood.

Toni and Lilia (2009) studied the erythrocytes of tropical marine fish *Bathygobius soporator.* The aim of study was to verify the efficiency of the micronucleus assay in field and laboratory work.

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The frequencies of micronuclei were compared with the samples in field and laboratory both. They found that micronucleus assay is quite efficient to assess the field pollution and laboratory. It has been reported by Cavas and Ergene-Gozukara, (2003) that micronucleus is formed either by small chromatin fragments which arise as a consequence of breakdown of chromosome after clastogenic action or by whole chromosome that do not move during anaphase due to aneugenic effects.

They showed micronuclei , nuclear lesions and interphase silver stained nuclear organizers regions (AgNos) as cyto-genotoxicity indicators in *Oreochromis niloticus* exposed to textile effluent.

Table showing some contributions regarding MN test in fishes

1	Boettcher *et al*. 2010	-	*Barbus barbus*
2	De-Lemos *et al*. 2001	-	The fathead minnow
3	Cavas and Ergene-Gezukara 2005	-	*Oreochromis niloticus*
4	Zhie *et al*. 2001	-	*Cyprinus Cario*
5	Obiokar *et al*. 2010a	-	*Synodontis clarias* and *Tilapia nilotica*
6	Obiokar *et al*. 2010c	-	*Clarias gariepinus*
7	Fagr. *et al*. 2008	-	*Oreochromis niloticus oreochromis aureus, Tilapia Zilli, Clarias gariepinus*
8	Palhares and Grisolia 2002	-	*Tilapia*
9	Rodriguez *et al*., 2003	-	*Salmo trutta*
10	Pandey *et al*. 2014	-	*Anguil anguilla, Phoxinus phoxinus, Channa punctatus*
11	Carlos Arbesto Machado Da Rocha *et al*. (2009)	-	*Colossoma macropomum*

Conclusion

As far as the author could hunt out from the present study it may be concluded that the MN test is the most widely used assay and an authentic tool for the assessment of genotoxicity in the aquatic environment because study of micronucleus frequencies proved to be very authentic for the assessment of genotoxicity in fishes. So, proper use of the assay may help in biomonitoring of the environment. Moreover, roads are always open for further investigation and research with special reference to fish because fishes play an important role in the trophic level of an ecosystem.

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