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Review Article

AN ANTHOLOGY OF CYTOGENETIC END POINTS LIKE MICRONUCLEUS TEST, COMET ASSAY AND CHROMOSOMAL ABERRATION ASSAY IN PISCES

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ABSTRACT. Growing awareness for chemicals with potential hazards has stimulated significant interest to use fishes as indicators for mutagens, carcinogens and teratogens in aquatic ecosystem as they take higher place in the food chain forming significant source of food and nutrition for human affecting health directly. They are generally considered as best animal model for studies with advantages of monitoring genotoxicity owing to their ability to metabolize xenobiotics and accumulate pollutants. This has led todevelop several biological tests to detect and identify such effectsthrough various cytogenetic endpoints which are very sensitive genetic assays to detect environmental mutagens at sub-toxic levels. Micronuclei tests (MN), comet assay and chromosomal aberrations test (CA) in fishes were reported as useful biomarker of in vivo techniques from time to time with potential for in situ monitoring of water quality. Various techniques of cytogenetic end point assay using fish in vitro and in vivo and their applications to environmental monitoring and eco genotoxicology has gained considerable significance from time to time. This article aims to present an anthology of such studies in piscine models to realize their significance in environmental mutagenesis. It justifies the use of piscine models in such studies like any other experimental animal model.

Keywords: *Fishes, Genetic Disorder, Hazards, Mutagens, Pollutants*

INTRODUCTION

Since last few decades, human civilization has been exposed to a very huge number of chemicals with potential hazards to genomeeither knowingly or inadvertently, while accomplishing all regular day to day activities. The industrial development and rapid urbanization has led to development of polluted zones discharging potentially toxic compounds in the environment. Quite a large number of chemical pollutants have proven record of potential hazards to affected organisms. Thousands of natural or synthetic materials in their ionic, simpler or complex molecular forms are used for different purposes in pharmaceuticals, drugs, beverages, cosmetics, perfumes, confectionaries, tobacco industries, food and food industries, food colorants, dyes, paints, enamels, cement, asbestos etc. and many more. These are added regularly to the environment in a pursuit to improve life style and living standard. In addition, various organic and inorganic effluents from different mills or industries, primary or secondary metabolites, heavy metals like Pb, Al, Cr, Ni, As, Sb, Zn, Sn, Hg and agrochemicals like fertilizers, pesticides, insecticides, herbicides increase the bulk content of this list of hazardous materials. Many of them can induce death of exposed organisms orcause physiological alterations or genetic disorders.

Mutagenicity induced by heavy metals even in sub-lethal concentrations can cause development of tumor [1] or undesirable alterations in genetic materials [2, 3]. Some of these chemicals in low concentrations may not cause acute detectable effects but in the

long run may reduce the life span [4] of the organisms exposed to them. Higher level of Pb and Hg can cause wide range of toxicity like muscular and neurological degeneration, destructive growth inhibition, mortality, reproductive problems and paralysis [5]. Various metallic ions act as genotoxins at particular concentrations due to their ability to bind to thiol groups and induce instability in the spindle formation in the cells [6]. Some heavy metals, such as copper, iron, nickel and chromium are importantmetals due to their essential functions in living systems, whereas cadmium and Pb are non-essential and are toxic even in trace amounts [7]. Certain agrochemicals inhibit cell division, induce chromosomal abnormalities and damage the genome [8]. This article aims to present an anthology of such studies in various fishes with particular reference to cytogenetic end points realizing their significance in environmental genotoxicity.

Why Piscine models?

Aquatic environment covers more than two-thirds of the earth inhabited by more than 28,000 fish species [9]. Growing awareness for the aquatic pollutant generated potential hazards has stimulated significant interest in their use as indicators for monitoring of environmental mutagens, carcinogens and teratogens [10]. They have been used in numerous biochemical and toxicological studies linked to development, carcinogenicity and teratogenicity both in vitro and in vivo [11, 12, 13] due to their easiness to handle and maintain ad libitum by relatively low cost methods [14]. Aquatic environment remains the ultimate recipient of an increasing number of agrochemicals where many of themare able to interact with DNA leading to gene mutation or genetic syndromes [15, 16] in aquatic organisms, particularly fishes. For different assay/analytical activities at genomic/cellular level, various species of fishes have been proved as suitable piscine model. Rodriguez-Cea et al. [17] noted that some fish species are more sensitive to genotoxic pollutants than other species such as eel (Anguilla anguilla) or minnow (Phoxinus phoxinus). As per Braunbeck et al. [18] and Osman et al. [19], they are considered to be efficient and cost effective as best toxicity indicators (Ruperelia et al. [20] and Barse et al. [21]) for evaluation of potentially teratogenic and carcinogenic substances [22]. At the top of the aquatic food chain they may directly affect human health by making them significant for their bio monitoring [23, 24]. Any change in the natural conditions of aquatic medium causes several physiological adjustments in them [25]. Fishes can take up both essential metals and nonessential metals which accumulate in their tissues [26]. As per Lavanya et al. [27], contaminants get accumulated in major aquatic organisms and Adeogun and Chukwuka [28] reported that, they are the final sink for many chemicals with long term effect on reproduction and gene pool of organisms. Rivero-Wendt et al. [29] proved lack of genotoxicity of 17α-Methyltestosterone (MT) in Oreochromis niloticus and Astyanax bimaculatus by cytogenetic studies. Some of their advantages as suitable model for monitoring aquatic genotoxicity owe to their ability to metabolize xenobiotics and accumulate pollutants [30, 31, 32]. Akpoilih [33] examined the use of ecogenotoxicology in environmental monitoring, the role of fish in genotoxicity testing of pollutants, genetic basis in genotoxicological assessment, current methods of ecogenotoxicological hazard assessment using fish in vitro and in vivo and their applications to environmental monitoring as well as recent advances in the field of fish eco genotoxicology and highlighted limitations and recommendations for further research on the use of eco genotoxicology.

Cytogenetic end points

Krishnaja and Rege [34] showed Boleophthalmus dussumieri as a satisfactory cytogenetic model in vivo for mutagenic studies. Manna [35] informed that, cytogenetic analysis constitutes important short term assay system for evaluation of genotoxic potentials of environmental agents which were applicable earlier to non piscine models formetaphase analysis [35], micronucleus test [36, 37], sister chromatid exchange analysis [38] and spermatocyte chromosome analysis of in vivo models. According to Manna [39], fishes are employed to assess the cytogenetic effects of environmental and manmade mutagens [40, 41, 42] in the aquatic ecosystems supported by Braunbeck et al. [18], Mitchell and Kennedy [43] and Akiyama et al. [44]. Gopal Krishna [45] reported on detection of damage due to genotoxicants, radiation, and apoptosiscausing depletion of fish resources could be addressed through cytogenetic end points. Chromosomal aberrations test (CA) and micronuclei tests (MN) in fishes were reported as useful biomarker of in vivo techniques [46, 47]. Alink et al. [48] reported that, Eastern mudminnow (Umbra pygmaea L.) exposed for 11 days to Rhine water had a significantly higher number of SCE and an increased comet tail length compared with control fish exposed to groundwaterand concluded that genotoxins are still present in the river Rhine, but the genotoxic potential has markedly decreased as compared. Genotoxic studies using cytogenetic analysis in fishes have been demonstrated by a number of workers [2, 46, 49, 50, 51, 52, 53]. Insecticides/pesticides lead to DNA damage in form of micronucleus formation, chromosome aberrations and mitotic aberrations [54, 55, 56]. Mahboob et al. [57] reported cytogenetic effect of heavy metal in Clarias gariepinus using the micronucleus test, chromosomal aberrations and sister chromatid exchange suggesting that $HgCl_2$ caused genotoxic effects in fish. For agrochemicals, Cypermethrin induced genotoxicity was studied by various cytogenetic end points in different organisms like Simoniello et al. [58] in Prochilodus lineatus evaluating DNA damage using alkaline comet assay, Ansari et al. [59] in Channa punctatus by MN studies, Rana [60] in Channa punctatus by chromosomes analysis; Rakesh [61] in Labeo rohita inducing chromosomal aberrations. Similarly, genotoxicity of Malathion was reported by various workers in fishes like Kumar et al. [62] in Channa punctatus by micronucleus and comet assay; Parveen and Shaadab [63] in the same species revealing clastogeneicity of chromosomes. Thus many chemicals were tested from time to time in different piscine models revealing the importance of cytogenetic end point analysis in genotoxicity studies.

a) Micronucleus test (MN)

The MN test, developed by Schmidt [64] is an in vivo and in vitro short time screening method widely used to detect genotoxic effects. These are cytoplasmic mass of chromatin with appearance of small nuclei arising from chromosomes lagging behind in anaphase. Using them, scientists evaluated potential clastogenicity of inhaled substances like cigarette smoke, methyl isocyanate, ozone and many other chemicals by analyzing bone marrow cells and blood lymphocytes of Chinese hamsters, rats and mice [65, 66, 67] and MN were first described in cytoplasm of erythrocytes as “fragment of nuclear material” by Howell or “intra-globularies corpuscles” in terminology of Jolly in late 18th century and early 1900 known to haematologists as Howell-Jolly bodies [68]. According to Heddle et al. [69], clastogenic and aneugenic agents are known to affect the spindle apparatus and can be differentiated on the basis of the relatively induced micronucleus sizes. Heddle and Salmone [70] described it as one of the simplest,

reliable, least expensive, sensitive and rapid screening system for both clastogenic and aneugenic effects [46, 71, 72] The micronucleus test in circulating erythrocytes of fish has been widely employed for both in situ exposure to environmental waters [73, 74, 75] and laboratory treatments in vivo [49, 76, 77, 78] in particular reference to Cyprinus carpio [79, 80, 81]. Their count has served as an index of chromosome break and mitotic spindle dysfunction [82]. It is widely employed to assess the biological impacts of aquatic pollutants [83, 84, 85] since it is associated with chromosome aberrations [86]. MN is the most widely used assay due to its proven correctness for fish [46]. Their presence in cells reflects structural and/or numerical chromosomal aberrations arising during mitosis [69, 87, 88] and its frequency is extensively used as a biomarker of genomic stability [88]. The mean frequencies of MN in piscivorous species have been shown to be almost five fold higher in the detrivorous and/or omnivorous species [89] and also reported significantly higher mean frequencies of MN in Prochilodus nigricans (detritivorous), Mylossoma duriventris (omnivorous) and Hoplias malabaricus (piscivorous) from the Madeira River compared to the frequencies observed in the same species in the Solimoes River. The MN has been employed successfully in various fish species to detect mutagenic changes caused by aquatic pollutants [90]. As per Fenech et al. [91]) and Udroiu [92] it is one of the most popular tests of environmental genotoxicity serving as an index of cytogenetic damage. According to Kirsch Volders et al. [93], the assay is a multi endpoint test of genotoxic responses to clastogens. The assay is sensitive for evaluating genotoxicity of compounds in fish [94] commonly used for the estimation of biological impacts of water pollutants in fish [95]. Their appearance in the cytoplasm is considered as biomarker of DNA damage [96]. Micronuclei are very small fragments of chromatin material developed from broken section of chromosome or from the chromosomes that could not be incorporated into daughter nuclei [97]. Erythrocytes of fish present a high frequency of MN and NAs after exposure to different heavy metals [46, 98, 99]. Their presence in cell reflects structural and/or numerical chromosomal aberrations [100]. The assay is an easy and ideal monitoring system to assess genotoxicity of water [101] allowing quick result for bio monitoring [84] of aquatic pollutants [102]. MN ensures continuous and effective evaluation of metallic pollution in aquatic environments [103]. MN is applied to evaluate genotoxicity of chemicals in fishes and their biological monitoring [104, 105].

Fig. 1. *Micronuclei induced infishes due to genotoxicity of chemicals*

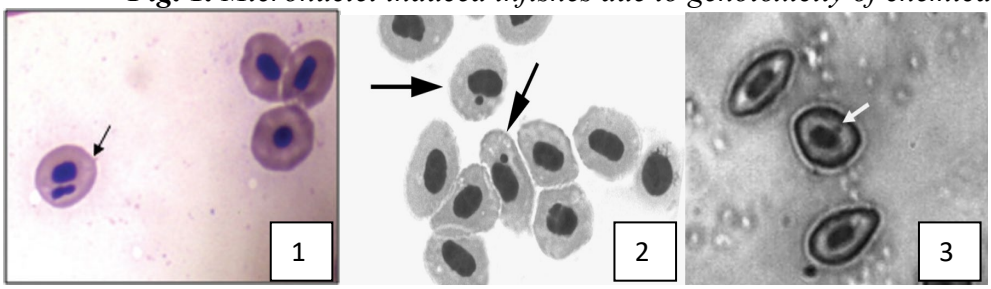

Plate 1: MN of Cirrhinus mrigala after exposure to Chlorpyrifos [106], **Plate 2:** MN of Heteropneustes fossilis induced by synthetic Sindoor [107], **Plate 3:** MN of C. macropomum treated with Methyl Mercury [108]

Table 1. *Micronuclei induced in different fishes*

Sl No	Source	Year	Species	Conclusion
1	Bahari et al. [109]	1994	Clarias gariepinus	Concentration and time dependent increase in frequency
2	Sandra et al. [110]	1996	Barbus plebejus	MN for in situ mutagens in freshwaters
3	Svobodova et al. [111]	1997	Cyprinus carpio	Malachite green induces MN
4	Campana et al. [112]	1999	Cheridon interruptus interruptus	Genotoxicity of pyrethroid lambda cyhalothrin
5	De Lemos et al. [113]	2001	Pimephales promelas	Significant induction of micronucleated erythrocytes exposed to chromium (VI)
6	Gustavino et al. [81]	2001	Cyprinus carpio	Dose dependent increase in MN due to X- rays
7	Ale et al. [114]	2004	Oreochromis niloticus	Evaluated genotoxic effect of $(NO_3)_2Pb$
8	Ferraro et al. [115]	2004	Hoplias malabaricus	Evaluated mutagenic potential of tributyltin (TBT) and inorganic lead(PbII)
9	Farah et al. [116]	2006	Channa punctatus	Possible anti mutagenic potential of ethanolic extract of neem leaves
10	Jiraungkoorskul et al. [117]	2007	O. niloticus, Poronotus triacanthus and Puntius altus.	Induction of MN and other nuclear abnormalities
11	Ali et al. [118]	2008	Channa punctatus	Increasing effect on MN frequency with concentration of Chlorpyrifos
12	Galindo and Moreira [119]	2009	Bathygobius soporator	Verified the efficiency of MN and NAs
13	Rocha et al.[108]	2009	Colossoma macropomum	MN and other NAs due to Methyl Mercury
14	Candioti et al. [120]	2010	Cnesterodon decemmaculatus	Genotoxicity of Aficida® by inducing MN
15	Ahmed et al. [121]	2011	Oreochromis mossambicus	Concentration dependent increase due to As
16	Ansari et al. [59]	2011	Channa punctatus	Cytogenetic effects of Cypermethrin using CA and MN
17	Ghisi et al. [122]	2011	Rhamdia quelen	MN and NAs due to Fipronil
18	Guner et al. [123]	2011	G. affinis	Significantly increased frequency of NAdue to Cu and Cd
19	Ansoar Rodriguez et al. [124]	2015	Oreochromis niloticus	Effect of Imidacloprid on genetic material using MN test and comet assay
20	Dar et al. [31]	2015	Carassius carassius (Cyprinidae)	Genotoxicity of Endosulfan by MN

MN: Micronuclei, CA; Chromosomal Aberration; NA: Nuclear Abnormalities, As: arsenic, Pb: Lead; Cu: Copper; Cd: Cadmium

b) Chromosomal aberration test (CA)

Chromosomal aberrations in fishes exposed to polluted aquatic environment were reported by several authors [125, 126, 127, 128, 129]. Carrasco et al. [130] reported formation of morphological nuclear abnormalities (NAs) in fish erythrocytes which includes lobbed (LB), blebbed (BL) and notched (NT) nuclei and bi nucleated (BN)

cells. According to Matter et al. [131], chromosomal aberration results from abnormalities in DNAduplication during S-phase. As per Das and John [132], genotoxic potential of methyl parathion and phosphamidon could be studied through induction of sister chromatid exchanges (SCE) and chromosome aberrations in gill tissues of Etroplus suratensis. Anitha et al. [133] showed the importance of aberration instudying the genotoxic effect of heat shock at different temperatures on gold fish Carassius auratus. Ouseph et al. [134] reported impact of physicochemical characteristics of river Cooum in Madras on the karyology of a native fish species Mystus vittatus where the species from the polluted river Cooum shows polyploidy, endo reduplication and condensed nature of chromosomal morphology causing irreparable damage to the genetic material of the fish as they are indicators of aquatic pollution. As per Mahrous and Abdou [135], water pollutants caused significant changesin chromosomal structures and centromeric attenuation in Oreochromis niloticus and Clarias lazera. Lopez-Poleza [136]) evaluated genotoxic effects of methyl mercury (CH_3Hg^+) in Hoplias malabaricus, using CA, MN and Comet assay. Cestari et al. [137] reported effects of clastogenic or mutagenic agents in neotropical fish Hoplias malabaricus using the comet (SCGE) assay and by testing for chromosomal aberrations showing that exposure leading significantly to increase frequency of chromosomal aberrations and the frequency of tailed cell nuclei indicating DNA damage. Chandra and Khuda-Bukhsh [138] studied the genotoxic effects of cadmium chloride ($CdCl_2$) and azadirachtin (Aza) singly and conjointly in a fish, Oreochromis mossambicus, with endpoints such as chromosome aberrations, abnormal red cell nuclei, abnormal sperm morphology and protein content. The bi-nucleation is an indicator of abnormal cell division due to blocking of cytokinesis resulting in genetic imbalance in the cells, may be involved in carcinogenesis [3]. Gadhia et al. [139] reported mitotic chromosomes from the gills of Boleophthalmus dussumieri for induction of CA after in vivo treatments with Bleomycin, Mitomycin-C and Doxorubicin revealing dose and time dependent increase in CA observing chromatid breaks, acentric fragments, dicentric and ring configurations. An increase in chromatid breaks andchromosomal exchange due to fluoride was reported by Chaurasia and Kumari [140]. Palikova et al. [141] reported genotoxicity of semi purified compound of microcystins and crude extract of cyanobacteria using detection of chromosomal aberrations in early life stages of weather fish revealing chromatid (gaps) and chromosomal aberrations (rings, dicentrics), percentage of which increased with the increased concentration of microcystins and the higher doses of crude cyanobacterial extract. Mohamed et al. [142] explored the capability of copper sulfate ($CuSO_4$) and lead acetate $(CHCOO)_3$ Pb in inducing chromosomal aberrations in aquatic organisms choosing Oreochromis niloticus and found that, effect of both chemicals on fish chromosomes and mitotic indices in gill cells displayed lower mitotic activity and positively induced macro-DNA damage represented by different types of aberrations e.g., chromatid deletions, chromatid breaks, gaps, fragments, stickness, translocations, ring chromosomes and centromeric attenuation. An increase in chromatid break and chromosomal exchange has been reportedby Rita and Milton [143] in Orechromis mosambicus on exposure to chromium. Kaur et al. [144] employed chromosomal aberration to study genotoxicity caused by dyeing industry effluent on a freshwater fish Cirrhinus mrigala and found chromosomal aberrations like chromosomal fragmentations (Cf), ring chromosomes (Rc), terminal chromatid deletions (Tcd), minutes (M), centromeric gaps (Cg), stickiness (Stk), clumping (C), pycnosis (Py), stretching (Stch) and pulverization (P). Yadav et al. [145] reported significant

frequencies of chromosomal aberration in a time dependent response in Cirrhinus mrigala exposed to Butachlor, showing stickiness and clumping of chromosomes demonstrating its genotoxic potential suggesting that, it interferes with cellular activities in fishes at genetic level inducing chromosomal aberrations. Promsid et al. [146], investigated chromosomal aberrations of snake head fish in a leachate-affected reservoir containing lead and mercury in water sediment observing four types of chromosomal breakages: single chromatid gap, isochromatid gap, single chromatid breaks and isochromatid breaks. Rana [60] revealed genotoxic potential of cypermethrin in Channa punctatus indicating the possibility of using fish chromosomes as indicators of genotoxic factors. Tengjaroenkul et al. [147] investigated chromosomal aberration in Rasbora tornieridue to arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb) contamination in water near gold mine area with higher chromosomal aberrations showing six types of chromosomal aberrations including centric fragmentation (CF), centric gap, single chromatid gap, fragmentation, deletion and polyploidy. Abd Ali et al. [148] described chromosomal aberration effects of electro fishing on Poecilia latipinna, located in Shat Al-Arab river in Al-garmma city (south of Iraq) showing decrease of mitotic index and significant increase in the most frequent aberration per 150 metaphase was chromosome break, fragment, range chromosome and the sticky chromosome mean were higher in comparison to non exposed organisms. Rakesh [61] carried cytogenetic study of Labeo rohita to check effects of lethal concentration (0.06 ppm) and acute lethal concentration (0.1 ppm) of Cypermethrin inducing chromosomal aberrations like acentric fragments, rings, double minutes and chromosome break, endo-reduplication, premature separation of chromosome and pulverizarion.

Fig. 2. *Chromosomal aberration in fishes induced by genotoxic chemicals*

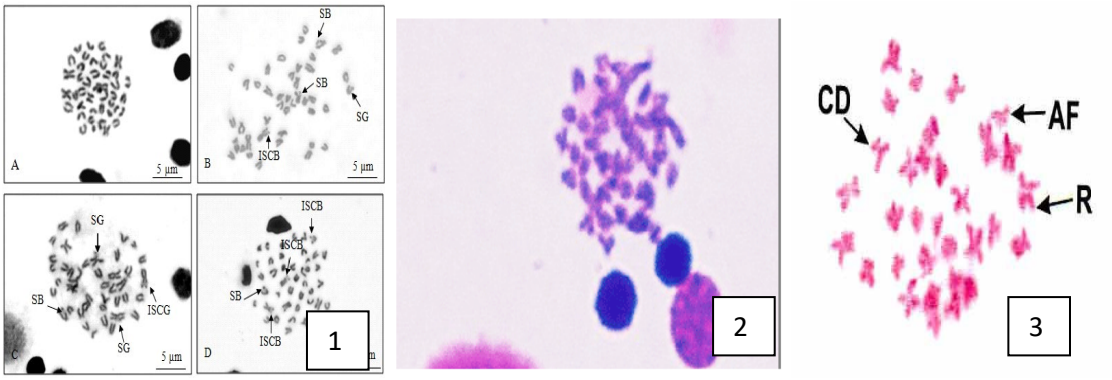

Plate-1: Chromosomal aberrations in C. striata like single chromatid gap (SG), isochromatid gap (ISCG), single chromatid breaks (SB) and isochromatid breaks (ISCB) affected by leachate of Pb and Hg [146]
Plate-2: A photomicrogragh shows a mitotic metaphase stage of O. niloticus with chromosomal fusion after treatment with Pb [142]
Plate-3: Metaphase spread with chromatid deletions (CD), acentric fragments (AF) and ring chromosomes (R) after treatment with Mitomycin-C in C. batrachus [149]

c) Single-cell gel electrophoresis (COMET) assay

The Comet assay was introduced by Ostling and Johanson [150] under neutral lysis and electrophoresis (pH 9.5) conditions as a result of studies undertaken to develop methodology of DNA electrophoresis in micro gel improved by Singh et al. [151] who launched the alkaline single cell gel electrophoresis (SCGE) based on principle of presence of single strand breaks of DNA fragments moving from the nucleoid core

towards the anode resulting in 'Comet' formation [152]. In this assay, cells are mixed with agarose and layered on microscope slides for lysis and electrophoresis; stained with fluorescent dyes i.e. DAPI or ethidium bromide for microscopic visualization of "Comets". DNA containing breaks unwinds and migrates away from the "head" (the nucleus), forming a "tail". Quantification of the amount of DNA in tails and in heads of provides an estimate of frequency of strand breaks. The molecular events that occur during processing of the cells and DNA to generate comets involve the DNA in chromatin arranged in "matrix attachment sites" and "loops" which are tightly supercoiled in undamaged dividing cells. One single-strand break is sufficient to release the superhelix tension in a loop, which is then free and can extend out from the nucleus. When the amount of damage is such that several loops have been affected, they form a "halo" that can be seen around the more intensely stained nucleus as stated by Mullenders et al [153]. Padrangi et al. [154] and Mitchelmore and Chipman [155] reported comet assay in red breast sunfish (Lepomis auritus), hard head cat fish (Anus felis), bullhead (Ameirurus nebulosus) and carp (C. carpio). DNA strand breakage could be detected by alkaline single cell gel electrophoresis (Comet assay) been applied in aquatic vertebrate and invertebrate [155, 156, 157, 158] also in several fishes showing sensitivity to genotoxic effects [155, 159, 160]. Lee and Steinert [161] informed that, exposure to genotoxins can damage the DNA of living cells and if these DNA lesions are not repaired, they can commence a cascade of biological consequences at the cellular, individual, community and finally at the population level. This has been employed since mid 1980s to study effects of environmental pollutants and occupational hazards, safety of therapeutic compounds, toxicology and to assess DNA repair capacity in human, animal and plant populations [162, 163] and to detect genetic damage in the form of DNA strand break in aquatic environments [158]. According to Ali et al. [118], Vanzella et al. [164] and Frenzilli et al. [165], comet assay has been successfully applied in many fish species exposed to different genotoxic agents, allowing the evaluation of DNA alterations with advantages like size and ploidy independency and mitotic activity is not a prerequisite as in metabolic rate and index in fish fluctuate considerably with temperature. de Campos Ventura et al. [166] showed that the assay in fishes is efficient to detect genotoxicity. In different modifications, the assay reflects variety of DNA damage in fish [167, 168, 169] and other aquatic animals [170, 171].

Comet assay has proved to be a useful tool for measuring the relationship between DNA damage and exposure of aquatic organisms to genotoxic pollutants [172] and considered more sensitive than cytogenetic techniques. According to Russo et al. [173] and Bucker et al. [174], MN is less sensitive than comet assay demonstrating genomic lesions that can be repaired reducing the number of stable lesions in DNA. Several international research groups have recommended protocols and criteria for comet assay, to establish high standards for valid, reproducible and accurate data [175, 176] increasingly used in testing of industrial chemicals, biocides, agrochemical, food additives and pharmaceuticals [176]. It is advantageous as per [118] due to its sensitivity for detecting low levels of DNA damage (0.1 DNA break/10^9 Daltons). According to Muid et al. [177], it is a suitable and rapid test for DNA damaging potential in environmental and biomonitoring studies. Nagarani et al. [178] reported utility of the assay for in vivo laboratory studies using fish.7

Table 2. *Comet Assay reported in different fishes to assess genotoxicity of chemicals*

Sl No	Source	Year	Species	Conclusion
1	Buschini et al. [179]	2004	Cyprinus carpio	Genotoxic damage due to water disinfected with sodium hypochlorite and chloride dioxide
2	Bucker et al. [174]	2006	Eingenmannia virescens	Benzene induced no significant results by MN but comet assay suggested genotoxicity in dose-dependent response
3	Vanzella et al. [164]	2007	Prochilodus lineatus	Genotoxicity of the soluble fraction of diesel (SFD) using the comet and MN
4	Wirzinger et al. [180]	2007	Gasterosteus aculeatus L.	Genotoxic potential of surface waters in Germany my MN and comet assay
5	Christofoletti et al.[181]	2008	Oreochromis niloticus	Methodological comparison of application of comet assay
6	Simoniello et al. [58]	2009	Prochilodus lineatus	Evaluated DNA damage using alkaline comet assay after in vivo exposure to Cypermethrin
7	Mitkovska et al. [182]	2017	Cyprinus carpio	Comet assay for in vitro exposure to heavy metals like Ni and Pb

MN: Micronuclei; SFD: Solid fraction of diesel; Ni: Nickel; Pb: Lead

Plate 1 **Plate 2** **Plate 3**

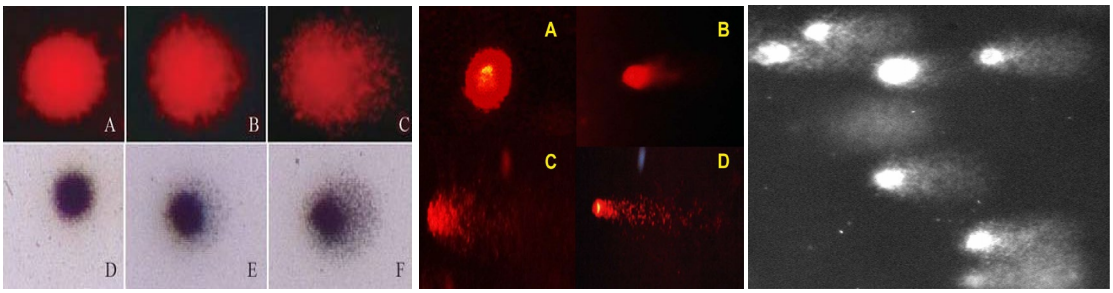

Fig. 3. *Comet test in different fish cells to reveal genotoxicity of pollutants*
Plate 1: In erythrocytes of O. niloticus using pH 12.1 stained with ethidium bromide and silver [181]
Plate 2: DNA damage in Catla catla exposed to chromium for different days [183]
Plate 3: Comets after single cell gel electrophoresis of gill cell DNA from Eastern mud minnows exposed to Rhine water [48]

Cytogenetic end point evaluations in fishes of India

Running parallel with technical advancements to assess the genotoxic potentials of various pollutants, Indian scientists were also well aware about the facts and employed fishes involving such techniques from time to time as evidenced from such vast array of references. Moorthy and Moorthy [184] analysed SCE, MN and CA in rodents exposed to mosquito coil smoke. Tripathy [185] reported that, CAs are quite significant in cytogenetic end point evaluations, including gap, chromatid break, fragments where gaps are achromatic lesion including unstained part of a chromatid appearing like an interruption; breaks are distinct dislocations of chromatid continuity and acentric fragments placed anywhere in the field of traceable origin or untraceable origin or some

available fine dots probably originated from terminal deletions. As per Bajpayee et al. [186], the comet assay is sensitive, rapid, and reliable method of quantitatively measuring DNA damage. According to Talapatra and Banerjee [187], detection of MN and NAs in fish helps us to assess the status of water quality. Sarangi [188] recommended use of MN in fish as sensitive indicator of aquatic pollution. Selection of peripheral blood erythrocytes of fish as target cell to investigate genotoxic damage is based on important role of blood in movement of toxic substances [189]. However, the anthology of references is still larger.

Table 3. *Cytogenetic assays in different fishes of India*

Sl No	Source	Year	Species	Conclusion
1	Manna et al. [190]	1985	Oreochromis mossambicus	MN induced by Aldrin, $CdCl_2$ and D-glucose amine hydrochloride as well as X-rays in fishes
2	Al Sabti [191]	1986 b	Cyprinus carpio, tench, Tinca tinca and grass carp	MN induced by aflatoxin B1, arochlor 1254, benzidene, benzo(a) pyrene and 20- methylchlo anthrene
3	Manna and Biswas [192]	1986	Labeo rohita, Catla catla, C.mrigala and O. mossambicus	MN in the blood smear of kidney and gill cells to assess the clastogenic potential of the bacterium Pseudomonas aeruginosa
4	Manna and Sadhukhan [193]	1986	Oreochromis mossambicus	MN in gill and kidney cells
5	Manna [194]	1989	Oreochromis mossambicus	Genotoxic potentiality through cytogenetic assays like somatic and germinal CA, mito-depression, MN, sperm head abnormality, dominant lethal test
6	Tripathy [195]	1993	Chela atapar, Mystus vittatus	Significant increase in incidence of MN in fishes exposed to paper mill effluent
7	Rishi and Sunita [51]	1995	Channa punctatus	Dichlorvos caused chromatid gaps, centromeric gaps, attenuation, chromatid breaks, extra fragments and stubbed arm
8	Ansy and Jahageerdar [2]	1999	C. punctata	Exposer to Pb induced CA
9	Sahoo and Bhunya [196]	2002	Heteropneustes fossilis	Carbaryl (Sevin ®) possess genotoxic potential
10	Farah et al. [197]	2003	Channa punctatus	Time dependent increase in the MN due to PCP and 2, 4–D Chlorpyrifos toxicity
11	Velmurugan et al. [198]	2006	Mystus gulio	Genotoxicity of pyrethroid pesticide lambda cyhalothrin by chromosomal aberration
12	Yadav and Trivedi [199]	2006	Channa punctata	Genotoxic potential of chromium [Cr (VI)] on aquatic biosystem causing chromatid breaks, chromosome breaks, chromatid deletions etc
13	Sharma et al. [200]	2007	Mystus vittatus	Single-cell DNA strand breaks induced by Endosulfan

14	Malla and Ganesh [201]	2009	H. fossilis	Increased incidence of CA including fragments and acrocentric associations
15	Tripathi et al. [149]	2009	Clarias batrachus	Fluoride is able to induce genotoxic effects in catfish
16	Yadav and Trivedi [202]	2009	Channa punctata	Chromosomal aberrations induced by heavy metals revealing chromatid and chromosome breaks, ring and di-centric chromosomes
17	Kumar et al. [62]	2010	Channa punctatus	Assessed genotoxic potential of Malathion using MN and comet assay
18	Nwani et al. [203]	2010	Channa punctatus	MN induction on exposure to Carbosulphan reporting concentration and duration dependency
19	Saxena and Chaudhuri [204]	2010	Channa punctatus	Exposure to Fenvalerate caused chromatid separation, chromatid break, deletion, fragments, gaps and ring type chromosomes
20	Yadav et al. [205]	2010	Cirrhinus mrigala	Significant frequencies of MN as a time dependent response to Butachlor observing broken egg (BE) and multiple micronuclei
21	Mohanty et al. [206]	2011	Labeo rohita	The phorate an organophosphate pesticide induces genotoxicity in fingerlings
22	Nwani et al. [207]	2011	C. punctatus	Exposer to Atrazine caused increase in DNA damage
23	Parveen and Shadab [63]	2011	C. punctatus	Clastogenicity of Malathion
24	Tahir et al. [107]	2011	H. fossilis	Genotoxicity of synthetic Sindoor
25	Kushwaha et al [208]	2012	C. punctatus, Mystus vittatus	Genotoxic potential of polluted water of river Gomti using MN and comet assay
26	Patowary et al. [209]	2012	C. punctatus	Significantly higher MN frequency due to arsenic exposure
27	Parveen and Shadab [210]	2012	C. punctatus	Genotoxic effect of heavy metal through MN, CA and SCE
28	Pavan et al. [211]	2012	C. punctatus	Significantly higher DNA damage in both lymphocyte and gill cells and micronuclei
29	Arunachalam et al. [183]	2013	Catla catla	Acute toxicity of chromium in fingerlings by MN and comet assay
30	Gadhave et al. [212]	2014	Labeo rohita	λ-cyhalothrin was genotoxic by MN assay
31	Ismail et al. [213]	2014	Labeo rohita	Chlorpyrifos is a genotoxic and neurotoxic insecticide causing DNA damage
32	Marques et al [214]	2014	Anguilla anguilla	Roundup® herbicide evaluated for genotoxicity
33	Chaudhari and Saxena [215]	2015	C. punctatus	Bifenthrin caused genotoxicity by using chromosomal aberration test

34	Nagpure et al. [216]	2015	Labeo rohita	Mutagenic and genotoxic effects of potassium dichromate by MN test and comet assay
35	Srivastava and Singh [217]	2015	Clarias batrachus	Genotoxic effects of Propiconazole by evaluating MN
36	Tripathi et al. [218]	2015	Labeo rohita	Genotoxic and mutagenic effects by formation of micronuclei, binucleated and multinucleated cells, pyknotic nucleus etc due to various chlorinated and phosphorylated insecticides/ pesticides and fertilizers
37	Bhatnagar et al. [106]	2016	Cirrhinus mrigala	Analyzed the incidence of NAs using MN assay due to acute toxicity of Chlorpyrifos
38	Rajan and Anandan [219]	2017	Clarias batrachus	Use of food colour containing Allura red and orange red inducing genotoxicity
39	Hussain et al. [220]	2018	Labeo rohita	Significant DNA fragmentation in river Chenab population
40	Tasneem and Yasmeen [221]	2018	Cyprinus carpio	Genotoxicity of sub lethal concentration of Karanjin

CONCLUSION

This present compilation of literary citations named as an anthology of cytogenetic end points like micronucleus test, comet assay and chromosomal aberration assay in pisces represents only a part of the huge references existing till to date in the same line and reflects the amicability and utility of different cytogenetic end point techniques as described above with particular reference to piscine models to test the genotoxic potential of various chemicals. Micronucleus test, comet assay and chromosomal abnormalities are truly significant assays in fishes to detect possible hazardous mutagens, carcinogens or teratogens in aquatic environment and the results are reproducible, replicable and reliable in fishes as well as in non piscine model organisms as per the need of the experiments. Their development, application and adaptation in different parts of the world as well as in Indian context are praiseworthy due to collective efforts of various individuals, research agencies or organizations with particular reference to fishes from time to time. These are significant to realize the genotoxic potential of various chemical pollutants in different fish models.

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REFERENCES

[1] Folmar, L. C. (1993): Effects of chemical contaminants on blood chemistry of teleostean fish: a bibliography and synopsis of selected effects. Environmental Toxicology and Chemistry, 12 (2): 337-375.

[2] Ansy, M. N. P., Jahageerdar, S. (1999): Effect of heavy metal on the karyotype of Channa punctatus. Indian Journal of Fisheries, 46: 167-72.

[3] Tolga, C. N., Garanko, N., Victor, V. A. (2005): Induction of micronuclei and binuclei in blood, gill and liver cells of fishes subchronically exposed to cadmium chloride and copper sulphate. Food Chemical Toxicology, 43(4): 569-74.

[4] Nehls, S., Segner, H. (2001): Detection of DNA damage in two cell lines from rainbow trout RTG-W1 using the comet assay. Environmental Toxicology, 16 (4):321–329.

[5] Tekale, N. S. (2003): Idol immersion: a critical analysis of environmental impacts on urban lakes and remedial measures. In: Souvenir on national Conference on Urban lakes-Environmental status, Economics and Management Options, Hyderabad, India, pp: 61-63.

[6] Patra, M., Bhowmik, N., Bandopadhyay, B., Sharma, A. (2004): Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environmental and Experimental Botany, 52 (3): 199-223.

[7] Fernandes, C., Fontainhas-Fernandes, A., Cabral, D., Salgado, M. (2008): Heavy metals in water, sediment and tissues of Liza saliens from Esmoriz-Paramos lagoon. Portugal Environment Monitoring Assesment, 136: 267-275.

[8] Silva, J., Heuser, V., Andrade, V. (2003): Biomonitoramento Ambiental. Genetica toxicologica, Alcance, Porto Alegre, 167-170.

[9] Nelson, J. S. (2006): Fishes of the World. 4th ed: John Wiley and Sons.

[10] Krishnaja, A. P., Rege, M. S. (1982): Induction of chromosomal aberrations in fish Boleophthalmus dussumieri after exposure in vivo to Mitomycin-C and heavy metals mercury, selenium and chromium. Mutation Research/Genetic Toxicology, 102(1):71-82.

[11] Nakatsuru, Y., Nemoto, N., Nakagawa, K., Masahito, P., Ishikawa, T. (1987): O-6 metylguanine DNA metyl transferase activity in liver from various fish species. Carcinogenesis, 8(8): 1123-1127.

[12] de Flora, S., Bagnasco, M., Zanacchi, P. (1991): Genotoxic, carcinogenic and teratogenic hazards in the marine environment with special reference to the Mediterranean Sea. Mutation Research, 258 (3): 285-320.

[13] Bailey, G., Hendricks, J., Dashwood, R. (1992): Anticarcinogenesis in fish. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 267(2): 243-250.

[14] Hayashi, M., Ueda, T., Uyeno, K., Wada, K., Kinae, N., Saotme, K., Tanaka, N., Takai, A., Sasaki, Y. F., Asano, N. et al. (1998): Development of genotoxicity assay systems that use aquatic organisms. Mutation Research, 399:125-133.

[15] Maccubbin, A. E., Ersing, N., Frank, M. E. (1991): Mutagenicity of sediments from the Detroit River. Journal of Great Lakes Research, 17(3):314-21.

[16] Kurelec, B. (1993): The genotoxic disease syndrome. Marine Environmental Research, 35(4):341-348.

[17] Rodriguez-Cea, A., Ayllon, F., Garcia-Vasquez, E. (2003): Micronucleus test in freshwater fish species: an evaluation of its sensitivity for application in field surveys. Ecotoxicology and Environmental Safety, 56(3):442–448.

[18] Braunbeck, T., Boettcher, M., Hollert, H., Kosmehl, T., Lammer, E., Leist, E., Rudolf, M., Seitz, N. (2005): Towards an alternative for the acute fish LC (50) test in chemical assessment: the fish embryo toxicity test goes multi-species- an update. Altex, 22(2):87-102.

[19] Osman, A. G. M., Wuertz, S., Mekkawy, I. A., Exner, H. J., Kirschbaum, F. (2007): Lead induced malformations in embryos of the African catfish Clarias gariepinus (Burchell, 1822). Environmental Toxicology, 22(4): 375–389.

[20] Ruperelia, S. G, Verma, Y., Hargan, M. C. (2001): Toxicity testing of effluents from pesticide industries using fish model. Ecology and Environment, 7:137-140.
[21] Barse, A. V, Chakrabarti, T., Ghosh, T. K., Pal, A. K., Jadhao, S. B. (2007): Endocrine disruption and metabolic changes following exposure of Cyprinus carpio to diethyl phthalate. Pesticides Biochemistry and Physiology, 88 (1):36-42.
[22] Matsumoto, S. T., Mantovani, M. S., Malagutti, M. I. A., Dias, A. L., Fonseca, I. C., Marin-Morales, M. A. (2006): Genotoxicity and mutagenicity of water contaminated with tannery effluents as evaluated by the micronucleus test and comet assay using the fish Oreochromis niloticusand chromosome aberrations in onion root-tips. Genetics and Molecular Biology,29(1):148–158.
[23] Zhou, Q., Zhang, J., Fu, J., Shi, J., Jiang, G. (2008): Biomonitoring: An appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal Chim Acta, 606 (2): 135-150.
[24] Foster, W. J., Chatelet, A. D. E., Rogerson, M. (2012): Testing benthic foraminiferal distributions as acontemporary quantitative approach tobiomonitoring estuarine heavy metal pollution. Marine Pollution Bulletin, 64:1039-1048.
[25] Garg, S., Gupta, R. K., Jain, K. L. (2009): Sub lethal effects of heavy metals on biochemical composition and their recovery in Indian major carps. Journal of Hazardous Materials, 163 (2-3): 1369-1384.
[26] Yılmaz, A. B., Sangun, M. K., Yaglıoglu, D., Turan, C. (2010): Metals (major, essential to non-essential) composition of the different tissues of three demersal fish species from Iskenderun Bay, Turkey. Food Chemistry, 123: 410-415.
[27] Lavanya, S., Ramesh, M., Kavitha, C., Malarvizhi, A. (2011): Hematological, biochemical and iono regulatory responses of Indian major carp Catla catla during chronic sub lethal exposure to inorganic arsenic. Chemosphere, 82: 977-985.
[28] Adeogun, A. O., Chukwuka, A. V. (2012): Toxicity of industrial waste water acting singly or in joint ratios on Clarias gariepinus. American Journal of Environmental Science, 8 (4): 366-375.
[29] Rivero-Wendt, C. L. G., Miranda-Vilela, A. L., Ferreira, M. F. N., Amorim, F. S., da Silva, V. A. G., Louvandini, H., Grisolia, C. K. (2013): Lack of genotoxicity in Astyanax bimaculatus and Oreochromis niloticus of 17 α-methyl testosterone used in fish hatcheries to produce male monosex populations. Genetics and Molecular Research, 12 (4): 5013-5022.
[30] Grisolia, C. K., Cordeiro, C. M. T. (2000): Variability in micronucleus induction with different mutagens applied to several species of fish. Genetics and Molecular Biology, 23 (1): 235–239.
[31] Dar, S. A., Yousuf, A. R., Balkhi, M. H., Ganai, F. A., Bhat, F. A. (2015): Assessment of endosulfan induced genotoxicity and mutagenicity manifested by oxidative stress pathways in freshwater cyprinid fish crucian carp (Carassius carassius L.). Chemosphere, 120: 273-283.
[32] Dar, S. A., Yousuf, A. R., Balkhi, M. H. (2016): An introduction about genotoxicology methods as tools for monitoring aquatic ecosystem: Present Status and Future Perspectives. Fisheries and Aquaculture Journal, 7 (1): 1-11.
[33] Akpoilih, B. U. (2012): Fish ecogenotoxicology: An emerging science, an emerging tool for environmental monitoring and risk assessment. Bulletin Environment, Pharmacology and Life Science, 2(1): 53- 64.
[34] Krishnaja, A. P, Rege, M. S. (1980): Some observation on the chromosome of certain teleosts using a simple method. Indian Journal of Experimental Biology, 18, 268-270.
[35] Manna, G. K. (1986): Mouse bone marrow as a mean of testing cytogenetic agents. Nucleus, 29:141-168.
[36] Das, R. K., Roy, B. B. (1988): A simplified method for micronucleus preparation from hepatic cells. Stain Technology, 63: 71-74.

[37] Roy, B., Das, R. K. (1990): Evaluation of genotoxicity of clofazimine, an antileprosy drug in mice in vivo II, Micronucleus test in bone marrow and hepatocytes. Mutation Research, 241: 169-173.
[38] Dash, B. C., Roy, B., Das, R. K. (1990): Evaluation of genotoxicity of Clofazimine, an antileprosy drug in mice in vivo III sister chromatid exchange analysis in bone marrow cells. In Vivo, 41.
[39] Manna, G. K. (1984): Progress in fish cytogenetics. The Nucleus, 27 (3): 203-231.
[40] Longwell, A. C., Hughes, J. B. (1980): Cytologic, cytogenetic and developmental state of Atlantic mackerel eggs from sea surface waters of the New York Bight and prospects for biological effect monitoring with ichthyoplankton. Rapp. P-V Reun. Cons. Int. Explor. Mer., 179: 275-276.
[41] Kligerman, A. D. (1982): Fishes as biological detectors of the effects of genotoxic agents. In: Mutagenecity, New Horizons in Genetic Toxicology, J. H. Heddle (ed.), New York, Academic Press.
[42] Manna, G. K. (1983): Cytogenetic studies on fishes and amphibia. In: Genetical researches in India. XV Int. Cong. Genet., Delhi, ICAR publication.
[43] Mitchell, S., Kennedy, S. (1992): Tissue concentrations of organochlorine compound in common seals from the coasts of Northern Ireland. Science of Total Environment, 115: 235-240.
[44] Akiyama, M., Oshima, H., Nakamura, M. (2001): Genotoxicity of mercury used in chromosome aberration tests. Toxicology in Vitro, 15 (4-5): 463-467.
[45] Gopal Krishna (2000): Genotoxicity and environmental monitoring. In: Training Manual of Summer School on Recent Advancement in Marine Biotechnology, 7-27 August, 2000. Organized by Fish Genetics and Biotechnology Division, Central Institute of Fisheries Education, ICAR, India: pp. 41-43.
[46] Al-Sabti, K., Metcalfe, C. D. (1995): Fish micronuclei for assessing genotoxicity in water. Mutation Research, 343 (2-3): 121-135.
[47] Kushwaha, B., Nagpure, N. S., Srivastava, S. K., Ravindra, K., Verma, M. S. (2003): Variation of micronuclei in peripheral blood cells of Channa punctatus. Indian Journal of Animal Science, 73 (10): 1192-1193.
[48] Alink, G. M., Quik, J. T. K., Penders, E. J. M., Spenkelink, A., Rotteveel, S. G. P., Maas, J. L., Hoogenboezem, W. (2007): Genotoxic effects in the Eastern mudminnow (Umbra pygmaea L.) after exposure to Rhine water, as assessed by use of the SCE and Comet assays: A comparison between 1978 and 2005. Mutation Research, 631 (2): 93–100. doi: 10.1016/j.mrgentox.2007.03.011.
[49] Das, R. K., Nanda, N. K. (1986): Induction of micronuclei in peripheral erythrocytes of fish Heteropneustes fossilis by mitomycin-C and paper mill effluent. Mutation Research, 175: 67-71.
[50] Al Sabti, K., Franko, M., Andrijanie, B., Kenz, S., Stegnar, P. (1994): Chromium induced micronuclei in fish. Journal of Applied Toxicology, 14 (5): 333-6.
[51] Rishi, K. K., Sunita, G. (1995): Chromosome aberration test for the insecticide, dichlorvos of fish chromosomes. Mutation Research, 344 (1-2): 1-4.
[52] Clarice, T. D. L., Patricia, M. R., Nara, R. T., Bernardoo, E. (2001): Evaluation of basal micronucleus frequency and hexavalent chromium effects in fish erythrocytes. Environmental Toxicology and Chemistry, 20(6): 1320-1324.
[53] Maples, N. L., Bain, L. J. (2004): Trivalent chromium alters gene expression in the mummichog (Fundulus heteroclitus). Environmental Toxicology and Chemistry, 23 (3): 626-631.
[54] Sankar, P., Telang, A. G., Manimaran, A. (2010): Curcumin protects against cypermethrin-induced genotoxicity in rats. Environmental Toxicology and Pharmacology, 30 (3): 289-291.

[55] Hussain, R., Mahmood, F., Khan, M. Z., Khan, A., Muhammad, F. (2011): Pathological and genotoxic effects of atrazine in male Japanese quail (Coturnix japonica). Ecotoxicology, 20: 1–8.
[56] Hussain, R., Mahmood, F., Khan, A., Javed, M. T., Rehan, S., Mehdi, T. (2012): Cellular and biochemical effects induced by atrazine on blood of male Japanese quail (Coturnix Japonica). Pesticides Biochemistry and Physiology, 103: 38–42.
[57] Mahboob, S., Al-Balwai, H. F. A., Al-Misned, F., Ahmad, Z. (2014): Investigation on thegenotoxicity of mercuric chloride to freshwater Clarias gariepinus. Pakisthan Veterinary Journal, 34(1):100-103.
[58] Simoniello, M. F., Gigena, F., Poletta, G., Loteste, A., Kleinsorge, E., Campana, M., Scagnetti, J., Parma, M. J. (2009): Alkaline comet assay for genotoxic effect detection in neotropical fish Prochilodus lineatus (Pisces, Curimatidae). Bulletin of Environmental Contamination Toxicology, 83:155–158.
[59] Ansari, R. A., Rahman, S., Kaur, M., Anjum, S., Raisuddin, S. (2011): In vivo cytogenetic and oxidative stress-inducing effects of cypermethrin in freshwater fish Channa punctata Bloch. Ecotoxicology and Environmental Safety,74 (1):150-156.
[60] Rana, R. (2016): Chromosomal abnormalities in Channa Punctatus exposed to Cypermethrin. International Journal of Engineering Science and Computing, 6: 1825-1826.
[61] Rakesh, P. (2018): Cypermethrin exposed chromosomal aberrations of Indian major carp. International Research Journal, 7(8): 16-20.
[62] Kumar, R., Nagpure, N. S., Kushwaha, B. et al. (2010): Investigation of the genotoxicity of Malathion to freshwater Teleost fish Channa punctatus (Bloch) using the micronucleus test and comet assay. Archieves of Environmental Contamination Toxicology, 58 (1): 123-130.
[63] Parveen, N., Shadab, G. G. H. A. (2011): Evaluation of micronuclei and haematological profiles as genotoxic assays in Channa punctatus exposed to Malathion. Intlernational Journal of Science and Nature, 2(3): 625- 631.
[64] Schmidt, W. (1975): The micronucleus test. Mutation Research, 31 (1): 9-15.
[65] Korte, A., Wager, H. M., Obe, G. (1981): Simultaneous exposure of Chinese hamsters to ethanol and cigarette smoke: Cytogenetic Aspects. Toxicology, 20: 237-246.
[66] Basler, A. (1982): SCEs in lymphocytes of rats after exposure in vivo to cigarette smoke or to cyclophosphamide. Mutation Research/Genetic Toxicology, 102 (2):137-143.
[67] Kar, R. N., Khan, K. A., Sethi, N. (1989): Genotoxicity studies on mice after short term inhalation exposure to methyl isocyanate. Cytobios, 59: 167-176.
[68] Kirsch Volders, M., Sofuni, T., Aardema, M., Albertini, S., Fenech M. et al. (2003): Report from the in vitro micronucleus assay working group. Mutation Research, 540 (2): 153-163.
[69] Heddle, J. A., Cimino, M. C., Hayashi, M., Romagna, F., Shelby, M. D., Tucker, J. D., Vanparys, Ph., Mac Gregor, J. T. (1991): Micronuclei as an index of cytogenetic damage: past, present, and future. Environmental and Molecular Mutagenesis, 18 (4): 277–291.
[70] Heddle, J. A., Salmone, M. F. (1981): Chromosomal aberrations and bone marrow toxicity. Environmental Health Perspectives. 39: 23-27.
[71] Landolt, M. L., Kocan, R. M. (1983): Fish cell cytogenetics: a measure of genotoxic effects of environmental pollutants. In: J. O. Nriagu (Ed.), Aquatic Toxicology, Wiley, New York, pp. 335–352.
[72] Mersch, J., Beauvais, M. N. (1997): The micronucleus assay in freshwater mussel, Dreissena polymorpha to in situ monitor genotoxicity in freshwater environments. Mutation Research, 393 (1-2): 141-149.
[73] Hose, J. E., Cross, J. N., Smith, S. G., Diehl, D. (1987): Elevated circulating erythrocyte micronuclei in fishes from contaminated sites of southern California. Marine Environmental Research, 22: 167–176.

[74] Gronlund, W. D., Chan, S. L., Mc Cain, B. B., Clark, R. C., Meyers, M. S., Stein, J. E., Brown, D. W., Landahl, J. T., Krahn, M. M., Varnasi, U. (1991): Multidisciplinary assessment of pollution at three sites in Long Island Sound. Estuaries, 14: 299–305.

[75] Hughes, J. B., Herbert, A. T. (1991): Erythrocyte micronuclei in winter flounder (Pseudopleuronectes americanus): result of fieldsurveys during 1980–1988 from Virginia to Nova Scotia and in Long Island Sound. Archieves of Environment Contamination Toxicology, 20: 474–479.

[76] Hooftman, R. N., de Raat, W. K. (1982): Induction of nuclear anomalies (micronuclei) in the peripheral blood erythrocytes of the eastern mud minnow Umbra pygmaea by ethylmethansulphonate. Mutation Research, 104 (1-3): 147–152.

[77] Schultz, N., Norrgren, L., Gawe, J., Johannisson, A., Medhage, O. (1993): Micronucleus frequency in circulating erythrocytes from rainbow trout (Oncorhynchus mykiss) subject to radiation, an image analysis and flow cytometric study. Comparative Biochemistry and Physiology, 105: 207–211.

[78] Ayllon, F., Garcia-Vasquez, E. (2000): Induction of micronuclei and other nuclear abnormalities in European minnow Phoxinus phoxinus and mollie Poecilia latipinna: an assessment of the fish micronucleus test. Mutation Research, 467 (2): 177–186.

[79] Al Sabti, K. (1986): Clastogenic effect of five carcinogenic mutagenic chemicals on the cells of the common carp Cyprinus carpio L. Comparative Biochemistry and Physiology, 85 (1):5–9.

[80] Nepomuceno, J. C., Ferrari, I., Spano, M. A., Centeno, A. J. (1997): Detection of micronuclei in peripheral erythrocytes of Cyprinus carpio exposed to metallic mercury. Environmental and Molecular Mutagenesis, 30 (3): 293–297.

[81] Gustavino, B., Scornajenghi, K. A., Minssi, S., Ciccoti, E. (2001): Micronuclei induced in erythrocyte of Cyprinus carpio (Teleostei, Pisces) by Xrays and colchicine. Mutation Research, 494: 151-159.

[82] Bombail, V., Gordon, E., Batty, J. (2001): Application of the comet and micronucleus assays to butterfish (Pholis gunnellus) erythrocytes from the Firth of Forth, Scotland. Chemosphere, 44 (3): 383–392.

[83] de Flora, S. L., Vigano, F., Agostini, A. D., Camoirano, M., Bagnasco, C., Bennecelli, F., Melodia, F., Arillo, A. (1993): Multiple genotoxicity biomarkers in fish exposed in-situ to polluted river water. Mutation Research, 319: 167–177.

[84] Minissi, S., Ciccoti, E., Rizzoni, M. (1995): Micronucleus test in erythrocytes of Barbus plebejus (Teleostei, Pisces) from two natural environments: A biossay for the in situ detection of mutagens in freshwater. Mutation Research/Genetic Toxicology, 367 (4):245-251.

[85] Vigano, L., Camoirano, A., Izzotti, A. A., Agostini, F. D., Polesello, S., Francisci, C., De Flora, S. (2002): Mutagenicity of sediments along the Po river and genotoxicity biomarkers in fish from polluted areas. Mutation Research, 515:125–134.

[86] Remirez, A., Saldanha, P. H. (2002): Micronuclei investigation of alcoholic patients with oral carcinomas. Genetics and Molecular Research, 1 (3): 246-260.

[87] Fenech, M., Holland, N., Chang, W. P., Zeiger, E., Bonassi, S. (1999): The human micronucleus project Dan international collaborative study on the use of micronucleus technique for measuring DNA damages in humans. Mutation Research, 428: 271-283.

[88] Norppa, H., Falck, G. C. M. (2003): What do human micronuclei contain? Mutagenesis, 18 (3): 221-233.

[89] Porto, J. I. R., Araujo, C. S. O., Feldberg, E. (2005): Mutagenic effects of mercury pollution as revealed by micronucleus test on three Amazonian fish species. Environmental Research, 97 (3): 87-92.

[90] Pantaleao, S. de M., Alcantara, A. V., Alves, P. J., Spano, M. A. (2006): The piscine micronucleus test to assess the impact of pollution on the Japaratuba River. Brazil Environmental and Molecular Mutagenesis, 47: 219-224.

[91] Fenech, M., Chang, W. P., Kirsch-Volders, M., Holland, N., Bonassi, S., Zeigere (2003): Human Micronucleus Project. "HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutation Research, 534 (1-2): 65-75.
[92] Udroiu, I. (2006): The micronucleus test in piscine erythrocytes. Aquatic Toxicology, 79(2): 201–204
[93] Kirsch Volders, M., Matenca, R. A., Roelants, M., Treamp, A., Zeigar, E. et al., (2006): The effect of GSIM 1 and GSTT 1 polymorphisms on micronucleus frequencies in human lymphocytes in vivo. Cancer Epidemiology, 17: 379-386.
[94] Bolognesi, C., Perrone, E., Roggieri, P., Pampanin, D. M., Sciutto, A. (2006): Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Aquatic Toxicology, 78(Suppl 1):93-98.
[95] Ergene, S., Cavas, T., Celik, A., Koleli, N., Kaya, F., Karahan, A. (2007): Monitoring of nuclear abnormalities in peripheral erythrocytes of three fish species from the Goksu Delta (Turkey): genotoxic damage in relation to water pollution. Ecotoxicology, 16:385-391.
[96] Saleh, K., Sarhan, M. A. A. (2007): Clastogenic analysis of chicken farms using micronucleus test in peripheral blood. Journal of Applied Science Research, 3: 1646–1649.
[97] Fagr, A., El-Shehawi, A. M., Seehy, M. A. (2008): Micronucleus test in fish genome: a sensitive monitor for aquatic pollution. African Journal of Biotechnology, 7: 606-612.
[98] Cavas, T., Ergene-Gozukara S. (2005): Micronucleus test in fish cells: a bioassay for in situ monitoring of genotoxic pollution in the marine environment. Environmental and Molecular Mutagenesis, 46: 64–70.
[99] Isani, G., Andreani, G., Cocchioni, F., Fedeli, D., Carpene, E., Falcioni, G. (2009): Cadmium accumulation and biochemical responses in Sparus aurata following sub-lethal Cd exposure. Ecotoxicology and Environmental Safety, 72: 224-230.
[100] Bolognesi, C., Hayashi, M. (2011): Micronucleus assay in aquatic animals. Mutagenesis, 26: 205–21.
[101] Bucker, A., Carvalho, M., Conceicao, M., Alves-Gomes, J. (2012): Micronucleus test and comet assay in erythrocytes of the Amazonian electric fish Apteronotus bonapartii exposed to benzene. Journal of Brazilian Society of Ecotoxicology, 7: 65-73.
[102] Saotome, K., Hayashi, M. (2003): Application of a sea urchin micronucleus assay to monitor aquatic pollution: influence of sample osmolality. Mutagenesis, 18 (1):73-76.
[103] Obiakor, M. O., Okonkwo, J. C., Nnabude, P. C., Ezeonyejiaku, C. D. (2012): Eco-genotoxicology: Micronucleus assay in fish erythrocytes as in situ aquatic pollution biomarker: a review. Journal of Animal Science Advances, 2: 123-133.
[104] Souza, T. S., Fontanetti, C. S. (2006): Micronucleus test and observation of nuclear alterations in erythrocytes of Nile Tilapia exposed to waters affected by refinery effluent. Mutation Research, 605: 87-93.
[105] Della Torre, C., Buonocore, F., Frenzilli, G., Corsolini, S., Brunelli, A., Guidi, P., et al. (2015): Influence of titanium dioxide nanoparticles on 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin bioconcentration and toxicity in the marine fish European Sea Bass (Dicentrarchus labrax). Environmental Pollution, 196: 185-193.
[106] Bhatnagar, A., Yadav, A. S., Cheema, N. (2016): Genotoxic effects of chlorpyrifos in freshwater fish Cirrhinus mrigala using micronucleus assay. Advances in Biology, Volume 2016: 1-6.
[107] Tahir M. M., Senthilkumar, C. S., Akhtar, S., Ganesh, N. (2011): Micronuclei as an evidence of DNA damage in freshwater catfish Heteropneustes fossilis (Bloch) exposed to synthetic sindoor. ARPN Journal of Agricultural and Biological Science, 6 (5): 41-44.
[108] Rocha, C. A. M., Santos, R. A., Bahia, M. D. O., Cunha, L. A. J. D., Ribeiro, H. F., Burbano, R. M. R. (2009): The micronucleus assay in fish species as an important tool for

xenobiotic exposure risk assessment-A brief review and an example using Neotropical fish exposed to Methyl mercury. Review of Fisheries Science, 17(4): 478–484.

[109] Bahari, I. B., Noor, F. M., Daud, N. M. (1994): Micro nucleated erythrocytes as an assay to assess actions by physical and chemical genotoxic agents in Clarias gariepinus. Mutation Research, 313:1-5.

[110] Sandra, S., Cicotti, E., Rizzoni, M. (1996): Micronucleus test in erythrocytes of Barbus plebejus (Teleostei, Pisces) from two natural environments; a bioassay for the in- situ detection of mutagens in freshwaters. Mutation Research, 367: 141-149.

[111] Svobodova, Z., Flaj Shan, M., Vykusova, B., Machova, J. (1997). The effect of long term therapeutic batch of malachite green on common carp (Cyprinus carpio). Acta Veterinaria Brno Acta Vet Brno. 66 (2): 111-117.

[112] Campana, M. A., Panzeri, A. M., Moreno, V. O., Dulout, F. M. (1999): Genotoxic evaluation of the parathyroid lambda- cyclothrin using the micronucleus test in erythrocytes of the fish Cheirdon interrtuptus interrtuptus. Mutation Research Genetics Toxicology Environmental Mutation, 438 (2):155-161.

[113] de Lemos, C. T., Rodel, P. M., Terra, N. R., Erdtmann, B. (2001): Evaluation of basal micronucleus frequency and hexavalent chromium effects in fish erythrocytes. Environmental Toxicology Chemistry, 20: 1320-1324.

[114] Ale, E., Fenocchio, A. S., Pastori, M. C., Ribeiro, C. O., Cestari, M. M., Zacharzewski, C. (2004): Evaluation of the effects of $(NO_3)_2Pb$ on Oreochromis niloticus (Pisces, Cichlidae) by means of cytogenetic techniques. Cytologia, 69(4): 453-458.

[115] Ferraro, M., Fenocchio, A., Mantovani, M., Ribeiro, C., Cestari, M. (2004): Mutagenic effects of tributyltin and inorganic lead (Pb II) on the fish H. malabaricus as evaluated using the comet assay and the piscine micronucleus and chromosome aberration tests. Genetics and Molecular Biology, 27:103–107.

[116] Farah, M. A., Ateeq, B., Ahmad, W. (2006): Anti mutagenic effect of neem leaves extract in freshwater fish, Channa punctatus evaluated by cytogenetic tests. Science of The Total Environ., 364 (13):200-214.

[117] Jiraungkoorskul, W., Kosai, P., Sahaphong, S., Kirtputra, P., Chawlab, J., Charucharoen, S. (2007): Evaluation of micronucleus test's sensitivity in freshwater fish species. Research Journal of Environmental Science, 1(2): 56-63.

[118] Ali, D., Nagpure, N. S., Kumar, S., Kumar, R., Kushwaha, B. (2008): Genotoxicity assessment of acute exposure of chlorpyrifos to freshwater fish Channa punctatus (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Chemosphere, 71:1823-1831.

[119] Galindo, T. P., Moreira, L. M. (2009): Evaluation of genotoxicity using the micronucleus assay and nuclear abnormalities in the tropical sea fish Bathygobius soporator (Valenciennes, 1837) (Teleostei, Gobiidae). Genetics and Molecular Biology, 32 (2): 394-398.

[120] Candioti, J. V., Soloneski, S., Larramendy, M. L. (2010): Genotoxic and cytotoxic effects of the formulated insecticide Aficida on Cnesterodon decemmaculatus (Jenyns, 1842) (Pisces: Poeciliidae). Mutation Research, 703: 180-186.

[121] Ahmed, M. K., Habibullah-Al-Mamun, M., Hossain, M. A., Arif, M., Parvin, E., Akter, M. S., Khan, M. S., Islam, M. M. (2011): Assessing the genotoxic potentials of arsenic in tilapia (Oreochromis mossambicus) using alkaline comet assay and micronucleus test. Chemosphere, 84: 143-149.

[122] Ghisi, N., Ramsdorf, W. A., Ferraro, M. V. M. et al. (2011): Evaluation of genotoxicity in Rhamdia quelen (Pisces, Siluriformes) after sub-chronic contamination with Fipronil. Environmental Monitoring Assessment, 180 (1-4): 589-599.

[123] Guner, U., Dilek, F., Muranl, G. (2011): Micronucleus Test, Nuclear Abnormalities and Accumulation of Cu and Cd on Gambusia affinis (Baird & Girard, 1853). Turkish Journal of Fisheries and Aquatic Science, 11: 615-622.

[124] Ansoar-Rodríguez, Y., Christofoletti, C., Marcato, A., Correia, J., Bueno, O., Malaspina, O., Fontanetti, C. (2015): Genotoxic potential of the insecticide Imidacloprid in a non-target organism (Oreochromis niloticus-Pisces). Journal of Environmental Protection, 6: 1360-1367.
[125] Al-Sabti, K., Kurelec, B. (1985). Induction of chromosomal aberrations in the mussel, Mytilus galloprovincialis. Bulletin of Environmental Contamination Toxicology, 35: 660-665.
[126] Kumari, S. A., Ramkumaran, S. (2006): Chromosomal aberrations in Channa punctatus from Hussainsagar Lake, Hyderabad (A. P). Indian Journal of fisheries, 53(3): 359-362.
[127] Hafez, A. M. (2009): Mugil cephalus Genome: A sensitive monitor for genotoxicity and cytotoxicity in aquatic environment. Australian Journal of Basic and Applied Science, 3(3): 2176-2187.
[128] Mahmoud, A., Mohamed, Z., Yossif, G., Sharafeldin, K. (2010): Cytogenetical studies on some River Nile species from polluted and nonpolluted aquatic habitats. Egyptian Academy Journal of Biological Science, 2(1): 1-8.
[129] Rose, M.H., Sudhakar, K., Sudha, P.N. (2010): Effect of water pollutants on the freshwater fish, Hypophthalmicthys molitrix. The Ecoscan, 4(1): 31-35.
[130] Carrasco, K. R., Tiburi, K. L., Myers, M. S. (1990): Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Canadian Journal of Fisheries and Aquatic Science, 47 (11): 2123-2136.
[131] Matter, E. E, ELserafy, S. S., Zowail, M. E. M., Awwad, M. H. (1992): Genotoxic effect of carbamyl insecticide (sevin) on the grass carp Ctenopharygodan idella (VAL). Egyptian Journal of Histology, 15 (1): 9-17.
[132] Das, P., John, G. (1999): Induction of sister chromatid exchanges and chromosome aberrations in vivo in Etroplus suratensis (Bloch) following exposure to organophosphorus pesticides. Toxicology Letters, 104: 111-116.
[133] Anitha, B., Chandra, N., Gopinath, P., Durairaj, G. (2000): Genotoxicity evaluation of heat shock in gold fish (Carassius auratus). Mutation Research/Genetic Toxicology Environmental Mutagenesis, 469(1):1-8.
[134] Ouseph, A., Sundarsanam, D., Nainar, A. M., Gandheeswari, P. (2000): Frequency of chromosomal aberration in fish inhabiting polluted ecosystem. Pollution Research Paper, 19 (1):123-128.
[135] Mahrous, K., Abdou, H. (2001): Cytogenetical studies on some Nile fish. Journal of Egyptian German Society of Zoological Histological Histochemistry and Genetics, 36:133-144.
[136] Lopez-Poleza, S. (2004): Evaluation of methyl mercury (CH_3Hg^+) effects in Hoplias malabaricus through the frequency of chromosome aberrations and of the micronucleus test and comet assay. Master's Dissertation. University of Parana, Brazil (2004).
[137] Cestari, M. M., Lemos, P. M. M., Ribeiro, C. A. O., Costa, J. R. M. A., Pelletier, E., Ferraro, M. V. M., Mantovani, M. S., Fenocchi, A. S. (2004): Genetic damage induced by trophic doses of lead in the neotropical fish Hoplias malabaricus (Characiformes, Erythrinidae) as revealed by the comet assay and chromosomal aberrations. Genetics and Molecular Biology, 27(2):
[138] Chandra, P., Khuda-Bukhsh, A. R. (2004): Genotoxic effects of cadmium chloride and azadirachtin treated singly and in combination in fish. Journal of Ecotoxicology and Environmental Safety, 58: 194-201.
[139] Gadhia, P. K., Gadhia, M., Georje, S., Vinod, K. R., Pithawala, M. (2008): Induction of chromosomal aberrations in mitotic chromosomes of fish Boleophthalmus dussumieri after exposure in vivo to antineoplastics Bleomycin, Mitomycin-C and Doxorubicin. Indian Journal of Science and Technology, 1 (7): 1-6.
[140] Chaurasia, O. P., Kumari, C. (2007): Genotoxic effect of ground water salts rich in fluoride. Cytologia, 72(2):141-144.

[141] Palikova, M., Rabova, M., Krejci, R., Navratil, S., Blaha L. (2007): Chromosomal aberrations in early embryos of weather fish (Misgurnus fossilis L.) exposed to crude Cyanobacterial extract and semi purified compound of Microcystins-a Pilot Study. Acta Veterinary Brno., 76: S55-S60.
[142] Mohamed, M. M., EL-Fiky, S. A., Soheir, Y. M., Abeer, A. I. (2008): Cytogenetic studies on the effect of copper sulfate and lead acetate pollution on Oreochromis niloticus fish. Asian Journal of Cell Biology, 3: 51-60.
[143] Rita, A. J. J., Milton, J. M. C. (2008): Karyomorphological analysis of the fresh water cichlid Oreochromis mossambicus (Peter) exposed to carbamate pesticide methomyl (Lannate). Journal of Advanced Zoology, 29(1):57-61.
[144] Kaur, H., Kalotra, R, Walia, G. K., Handa, D. (2013): Genotoxic effects of dyeing industry effluent on a freshwater fish, Cirrhinus mrigala by chromosomal aberration test. International Journal of Pharmacy and Biological Science, 3 (1): 423-431.
[145] Yadav, A. S., Bhatnagar, A., Kaur, M. (2013): Aberrations in the chromosomes of Cirrhinus mrigala (Hamilton) upon exposure to Butachlor. Iranian Journal of Toxicology, 7 (21): 857-865.
[146] Promsid, P., Neeratanaphan, L., Supiwong, W., Sriuttha, M., Tanomtong, A. (2015): Chromosomal aberration of snakehead fish (Channa striata) in affected reservoir by Leachate with Lead and Mercury contamination. International Journal of Environmental Research, 9(3):897-906.
[147] Tengjaroenkul, B., Intamat, S., Boonmee, S., Neeratanaphan, L. (2017): Chromosomal aberration assessment of silver rasbora fish (Rasbora tornieri) living near gold mine area with heavy metal contamination. Human Ecology Risk Assessment: An International Journal, 23 (5): 1140-1152.
[148] Abd Ali, M. A., Mohammed, M. H., Sadeq, M. K. (2018): In vitro chromosomal aberration frequency by electro fishing on Poecilia latipinna (Sailfin Molly) fishes in Southern of Iraq. American Journal of Molecular Biology, 8: 109-118.
[149] Tripathi, N., Bajpai, S., Tripathi, M. (2009): Genotoxic alterations induced by fluoride in Asian catfish, Carias batrachus (Linn.). Research Report Fluoride, 42(4):292–296.
[150] Ostling, O., Johanson, K. J. (1984): Microelectrophoretic study of radiation induced DNA damages in individual mammalian cells. Biochemistry and Biophysics Research Communication, 123: 291-298.
[151] Singh, N. P., Mc Coy, M. T., Tice, R. R., Schneider, E. L. (1988): A simple technique for quantitation of low levels of DNA damage in individual cells. Experimental Cell Research, 75:184-191.
[152] Park, J. K, Lee, H. H, Choi, I. S., Park, S. D. (1991): Accumulation of polyclic aromatic hydrocarbon-induced single-strand breaks is attributed to slower rejoining process by DNA polymerase inhibitor, cystosine arabinoside in CHO-Kl. Life Science, 48:1255-1261.
[153] Mullenders, L., van Zeeland, A., Natarajan, A. (1987): The localization of ultraviolet induced excision repair in the nucleus and the distribution of repair events in higher order chromatin loops in mammalian cells. Journal of Cell Science Supplements, 6:243–262.
[154] Padrangi, R., Petras, M., Ralph, S., Vrzoc, M. (1995): Alkaline single cell gel (comet) assay and genotoxicity monitoring using bullheads and carp. Environmental and Molecular Mutagenesis, 26:345–356.
[155] Mitchelmore, C. L., Chipman, J. K. (1998): Detection of DNA strand breaks in brown trout (Salmo trutta) hepatocytes and blood cells using the single cell gel electrophoresis (Comet) assay. Aquaculture Toxicology, 41:161–182.
[156] Belpaeme, K., Delbeke, K., Zhu, L., Kirsh-Volders, M. (1996): Cytogenetic studies of PCB77 on brown trout (Salmo trutta fario) using the micronucleus test and the alkaline comet assay. Mutagenesis, 11:485–492.

[157] Nacci, D. E., Cayula, S., Jackim, E. (1996): Detection of DNA damage in individual cells from marine organisms using the single cell gel assay. Aquaculture Toxicology, 35: 197–210.
[158] Kleinjans, J. C. S., Van Schooten, F. J. (2002): Ecogenotoxicology: the evolving field. Environmental Toxicology and Pharmacology, 11: 173–179.
[159] Belpaeme, K, Cooreman, K., Kirsch-Volders, M. (1998): Development and validation of the in vivo alkaline comet assay for detecting genomic damage in marine flatfish. Mutation Research/ Genetics Toxicology and Environmental Mutagenesis, 415:167–184.
[160] Gontijo, A. M. M. C., Barreto, R. E., Speit, G., Reyes, V. A. V., Volpato, G. L., Salvadori, D.M.F. (2003): Anesthesia of fish with benzocaine does not interfere with comet assay results. Mutation Research, 534:165–172.
[161] Lee, R., Steinert, S. (2003): Use of the single cell gel electrophoresis /Comet assay for detecting DNA damage in aquatic (Marine and Freshwater) animals. Mutation Research/ Reviews in Mutation Research, 544: 43-64.
[162] Moller, P. (2005): Genotoxicity of environmental agents assessed by the alkaline comet assay. Basic and Clinical Pharmacology and Toxicology, 96:1–42.
[163] Moller, P. (2006): The alkaline comet assay: towards validation in biomonitoring of DNA damaging exposures. Basic and Clinical Pharmacology and Toxicology, 98:336–345.
[164] Vanzella, T. P., Colus Martinez, C. B., M. S. I. (2007): Genotoxic and mutagenic effects of diesel oil water soluble fraction on a neotropical fish species. Mutation Research, 631:36–43.
[165] Frenzilli, G., Nigro, M., Lyons, B. P. (2009): The comet assay for the evaluation of genotoxic impact in aquatic environments. Mutation Research, 681:80–92.
[166] de Campos Ventura, B., de Angelis, D., Marin-Morales, M. A. (2008): Mutagenic and genotoxic effects of the Atrazine herbicide in Oreochromis niloticus (Perciformes: Cichlidae) detected by the micronuclei test and the comet assay. Pesticides Biochemistry and Physiology, 90:42–51.
[167] Toyoizumi, T., Deguchi, Y., Masuda, S., Kinae, N. (2008): Genotoxicity and estrogenic activity of 3, 3'-dinitrobisphenol A in gold fish. Bioscience, Biotechnology and Biochemistry, 72: 2118-2123.
[168] Cavalcante, D. G., Martinez, C. B., Sofia, S. H. (2008): Genotoxic effects of round up on the fish Prochilodus lineatus. Mutation Research, 655: 41-46.
[169] Caliani, I., Porcelloni, S., Mori, G., Frenzilli, G., Ferraro, M., Marsili, L., Casini, S., Fossi, M. C. (2009): Genotoxic effects of produced waters in mosquito fish (Gambusia affinis). Ecotoxicology, 18:75-80
[170] Petridis, P., Jha, A. N., Langston, W. J. (2009): Measurements of the genotoxic potential of (xeno-) oestrogens in the bivalve mollusc Scrobicularia plana using comet assay. Aquaculture Toxicology, 94: 8-15.
[171] Binelli, A., Cogni, D., Parolini, M., Riva, C., Provini, A. (2009): In vivo experiments for the evaluation of genotoxic and cytotoxic effects of triclosan in Zebra mussel hemocytes. Aquaculture Toxicology, 91: 238-244.
[172] de Andrade, V. M., de Freitas, T., da Silva, J. (2004): Comet assay using mullet. (Mugil sp.) and sea catfish (Netuma sp.) erythroyites for the detection of genotoxic pollutants in aquatic environment. Mutation Research, 560:57–67.
[173] Russo, C., Rocco, L., Morescalchi, M., Stingo, V. (2004): Assessment of environmental stress by the micronucleus test and the comet assay on the genome of teleost populations from two natural environments. Ecotoxicology and Environmental Safety, 57:168–174.
[174] Bucker, A., Carvalho, W., Alves-Gomes, J. (2006): Evaluation of mutagenicity and genotoxicity in Eingenmannia virescens (Teleostei: Gymnotiformes) exposed to benzene. Acta Amazonica, 36: 357–230.
[175] Klaude, M., Eriksson, S., Nygren, J., Ahnstrom, G. (1996): The comet assay: Mechanisms and technical considerations. Mutation Research, 363:89-96.

[176] Brendler Schwaab, S., Hartman, A., Pfuhler, S., Speit, G. (2005): The in vivo comet assay: use and status in genotoxicity testing. Mutagenesis, 20:245–254.
[177] Muid, K. A., Shahjahan, R. M., Begum, R., Begum, R. A. (2012): Zinc phosphide induced DNA damage in the blood cells of Gallus sp. using comet DNA assay. American Journal of Agricultural and Biological Science, 7: 82-87.
[178] Nagrani, N., Janaki Devi, V., Kumaraguru, A. K. (2012): Identification of DNA damage in marine fish Therapon jarbua by comet assay technique. Journal of Environmental Biology, 33: 699-703.
[179] Buschini, A., Martino, A., Gustavino, B., Monfrinotti, M., Poli, P., Rossi, C., Santoro, M., Dorr, A. J. M., Rizzoni, M. (2004): Comet assay and micronucleus test in circulating erythrocytes of Cyprinus carpio specimens exposed in situ to lake waters treated with disinfectants for potabilization. Mutation Research, 557: 119–129.
[180] Wirzinger, G., Weltje, L., Gercken, J., Sordyl, H. (2007): Genotoxic damage in field-collected three-spined sticklebacks (Gasterosteus aculeatus L.): A suitable biomonitoring tool? Mutation Research, 628: 19–30.
[181] Christofoletti, C. A., David, J. A. O., Fontanetti, C.S. (2008): Application of the comet assay in erythrocytes of Oreochromis niloticus (Pisces): A methodological comparison**.** Genetics and Molecular Biology, 32(1): 155–158.
[182] Mitkovska, V. I., Dimitrov, H. A., Chassovnikarova, T. G. (2017): In vitrogenotoxicity and cytotoxicity assessment of allowable concentrations ofnickel and lead: comet assay and nuclear abnormalities in acridine orangestained erythrocytes of common carp (Cyprinus carpio L.) Acta Zoologica Bulgarica, 8: 47–56.
[183] Arunachalam, K. D., Annamalai, S. K., Kuruva, J. K. (2013): In-Vivo evaluation of hexavalent chromium induced DNA damage by alkaline comet Assay and oxidative stress in Catla catla. American Journal of Environmental Science, 9 (6): 470-482.
[184] Moorthy, M. V., Moorthy, P. B. (1994): Analysis of sister chromatid exchange, micronucleus and chromosomal aberrations frequencies in rodents exposed to mosquito coil smoke by inhalation route. Toxicology Letters, 70:357-362.
[185] Tripathy, S. K. (1996): Evaluation of genotoxic effects of inhalation of petroleum vapour on pulmonary alveolar macrophase chromosomes of rats. In: M Phil. dissertation thesis submitted to Sambalpur University in Life Sciences. Orissa, India, Pp: 18.
[186] Bajpayee, M., Pandey, A. K., Parmar, D., Marthur, N., Seth, P. K., Dhawan, A. (2005). Comet assay responses in human lymphocytes are not influenced by the menstrual cycle: a case study in healthy Indian females. Mutation Research, 565:163–172.
[187] Talapatra, S. N., Banerjee, S. K. (2007): Detection of micronucleus and abnormal nucleus in erythrocytes from the gill and kidney of Labeo bata cultivated in sewage-fed fish farms. Food Chemistry Toxicology, 45: 210-215.
[188] Sarangi, P. K. (2012): Micronucleus assay: a sensitive indicator for aquatic pollution. International Journal of Research in Bio Science, 1 (2): 32-37.
[189] Kousar, S., Javed, M. (2015): Studies on induction of nuclear abnormalities in peripheral blood erythrocytes of fish exposed to copper. Turkish Journal of Fisheries and Aquatic Science, 15(4): 879-886.
[190] Manna, G. K., Banerjee, G., Gupta, S. (1985): Micronucleus test in the peripheral erythrocytes of the exotic fish Oreochromis mossambica. The Nucleus, 28 (3): 176-179.
[191] Al Sabti, K. (1986 b): Comparative micronucleated erythrocyte cells induction in three cyprinids by five carcinogenic, mutagenic chemicals. Cytobios, 47: 147-154.
[192] Manna, G. K., Biswas, S. (1986): Micronucleus test in four species of fishes treated with the bacterium Pseudomonas aeruginosa. National Academy of Science Letters, 9 (6): 189-191.
[193] Manna, G. K., Sadhukhan, A. (1986): Use of cells of gills and kidney of tilapia fish in micronucleus test (MNT). Current Science, 55 (10): 498-501.

[194] Manna, G. K. (1989): Cytogenetical assays of some toxicants using fish as a model. In: Harmful Effects of Common Environmental Toxicants, by Om Prasad (ed.), Zoology Deptt. University of Allahabad, Allahabad. Pp. 65-74.
[195] Tripathy, A. P. (1993): Induction of micronuclei in the peripheral blood of fish exposed to paper mill effluent in natural and laboratory conditions. M.Phil dissertation submitted to Sambalpur University, Orissa, India in Environmental Sciences, 1993: Pp. 25.
[196] Sahoo, S. N., Bhuyan, S. P. (2002): Evaluation of genotoxic potential of an insecticide (Sevin ®) in fish in vivo test system. In: Proc. Fifth Indian Fisheries Forum. Published by AFSIB, Mangalore and AoA, Bhubaneswar, India: pp. 219-221.
[197] Farah, M. A., Ateeq, B., Ali, M. N., Ahmad, W. (2003): Evaluation of genotoxicity of PCP and 2,4-dichlorophenoxyacetic acid by micronucleus test in fresh water fish Channa punctatus (Bloch). Environmental Toxicology and Pharmacology, 54 (1): 25–29.
[198] Velmurugan, B., Ambrose, T., Selvanayagam, M. (2006): Genotoxic evaluation of lambda-cyhalothrin in Mystus gulio. Journal of Environmental Biology, 27(2): 247-250.
[199] Yadav, K. K., Trivedi, S. P. (2006): Evaluation of genotoxic potential of chromium (VI) in Channa punctata fish in terms of chromosomal aberrations. Asian Pacific Journal of Cancer Prevention, 7: 472-476.
[200] Sharma, S., Nagpure, N. S., Kumar, R. et al. (2007): Studies on the genotoxicity of Endosulfan in different tissues of fresh water fish Mystus vittatus using the Comet assay. Archieves of Environmental Contamination Toxicology, 53 (4): 617-623.
[201] Malla, T. M., Ganesh, N. (2009): Cytogenetic and tissue toxicity by synthetic sindoor in freshwater catfish Heteropneustes fossilis. Biomed Pharma Journal, 2 (1): 77-81.
[202] Yadav, K. K., Trivedi, S. P. (2009): Chromosomal aberrations in a fish Channa punctata after in vivo exposure to three heavy metals. Mutation Research/Genetics Toxicology and Environmental Mutagenesis, 678(1):7-12.
[203] Nwani, C. D., Lakra, W. S., Nagpure, N. S., Kumar, R., Kushwaha, B., Srivastava, S. K. (2010): Mutagenic and genotoxic effects of carbosulfan in freshwater fish Channa punctatus (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. Food and Chemical Toxicology, 48 (1):202–208.
[204] Saxena, K. K., Chaudhari, R. (2010): Study of chromosomal abnormalities in C. punctatus expose to fenvalerate. Journal of Applied and Natural Sciience, 2: 70-73.
[205] Yadav, A. S., Bhatnagar, A., Kaur, M. (2010): Assessment of genotoxic effects of Butachlor in fresh water fish, Cirrhinus mrigala (Hamilton). Research Journal of Environmental Toxicology, 4 (4): 223-230.
[206] Mohanty, G., Mohanty, J., Nayak, A. K., Mohanty, S. and Dutta, S. K. (2011). Application of Comet assay in the study of DNA damage and recovery in rohu (Labeo rohita) fingerlings after an exposure to Phorate an Organophosphate pesticide. Ecotoxicology, 20: 283-292.
[207] Nwani, C. D., Nagpure, N. S., Kumar, R., Kushwahab, B., Kumar, P., Lakra, W. S. (2011): Mutagenic and genotoxic assessment of Atrazine based herbicide to freshwater fish Channa punctatus (Bloch) using micronucleus test and single cell gel electrophoresis. Environmental Toxicology and Pharmacology, 31 (2):314-322.
[208] Kushwaha, B., Pandey, S., Sharma, S., Srivastava, R., Kumar, R., Nagpure, N. S., Dabas, A., Srivastava, S. K. (2012): In situ assessment of genotoxic and mutagenic potential of polluted river water in Channa punctatus and Mystus vittatus. International Aquaculture Research, 4 (16): 1-11.
[209] Patowary, K., Hazarika, N. S., Goswami, M. (2012): Studies on the toxic impact of arsenic on some enzymes and chromosomes of Channa punctatus. The Clarion, 1: 148-153.
[210] Parveen, N., Shadab, G. G. H. A. (2012): Cytogenetic evaluation of cadmium chloride on Channa punctatus. Journal of Environmental Biology, 33 (3): 663-666.PMID: 23029919
[211] Pavan, K., Ravindra, K., Nagpure, N. S., Nautiyal, P., Dabas, A., Kushwaha, B., Lakra, W. S. (2012): Genotoxic and mutagenic assessment of hexavalent chromium in fish

following in vivo chronic exposure. Human Ecology Risk Assessment: An International Journal, 18(4):855-870.

[212] Gadhave, P. D., Brar, R. S., Banga, H. S., Dhawan, A. (2014). λ- cyhalothrin induced genotoxicity in freshwater fish Labeo rohita. Veterinary World, 7(6):412-415.

[213] Ismail, M., Qaiser, M. K., Ali, R., Ali, T (2014): Genotoxicity of chlorpyrifos in freshwater fish Labeo rohita using alkalines single-cell gel electrophoresis (Comet) assay. Drugs and Chemicals Toxicology, 37 (4):466-471.

[214] Marques, A., Guilherme, S., Gaivao, I., Santos, M. A., Pacheco, M. (2014): Progression of DNA damage induced by a Glyphosate based herbicide in fish (Anguilla anguilla) upon exposure and post exposure periods, Insights into the mechanisms of genotoxicity and DNA repair. Comparative Biochemistry and Physiology, Part C, 166: 126-133.

[215] Chaudhari, R., Saxena, K. K. (2015): Evaluation of Bifenthrin genotoxicity on Channa punctatus. International Journal of Pure and Applied Zoology, 3 (4): 297-301.

[216] Nagpure, N. S., Srivastava, R., Ravindra, K., Kushwaha, B., Srivastava, S. K., Pavan, K., Dabas, A. (2015): Assessment of genotoxic and mutagenic potential of hexavalent chromium in the freshwater fish Labeo rohita (Hamilton, 1822). Drugs and Chemicals Toxicology, 38 (1):9-15.

[217] Srivasatava, P., Singh, A. (2015): Evidence of micronuclei in fish blood as a biomarker of genotoxicity due to surface run off agricultural fungicide (Propiconazole). Journal of Toxicology and Environmental Health Sciences, 7 (1): 4-8.

[218] Tripathi, M., Mishra, R. P., Girdoniya, V. (2015): Study of the genotoxic effects of polluted water of certain ponds of Narsinghpur in the R.B.Cs. of the fish Labeo rohita by applying micronucleus assay. International Journal of Pharmaceutical Biological Archieves, 6(2): 9- 13.

[219] Rajan, A. P., Anandan, S. (2017): Investigation of carcinogenic and mutagenic property of food color using cat fish Clarias batrachus by using alkaline single-cell gel electrophoresis (COMET) assay and micronucleus assay. International Journal of Medical Research and Pharmaceutical Science, 4 (7): 29-34.

[220] Hussain, B., Sultana, T., Sultana, S., Masoud, M. S., Ahmed, Z., Mahboob, S. (2018). Fish eco-genotoxicology: Comet and micronucleus assay in fish erythrocytes as in situ biomarker of freshwater pollution. Saudi Journal of Biological Science, 25(2):393-398.

[221] Tasneem, S., Yasmeen, R. (2018): Evaluation of genotoxicity by comet assay (single-cell gel electrophoresis) in tissues of the fish Cyprinus carpio during sub-lethal exposure to Karanjin. Journal of Basic and Applied Zoology, 79 (19): 1-13.