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Review Article

An Overview of Environmental Contamination of Chromium and Micronuclei Formation in Fishes

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ABSTRACT

Heavy metals are cornerstones of human progress. They are quite literally the pillars of all major civilizations, past and present, on the one hand, on the other hand, they are also the environmental pollutants of global concern because of their non-biodegradability. Chromium, the earth's 6th most abundant heavy metal, is a well-known carcinogenic and mutagenic metal that is discharged into aquatic environments. With regard to toxicity, carcinogenicity, genotoxicity, and hexavalent chromium are considered the most potent one. Genotoxic pollutants interfere with the key functions of life since they affect the primary biological information matrix DNA. These pollutants have led to the development of several genotoxicological tests, namely, chromosomal aberration test, micronucleus (MN) test, sister chromatid exchange, and single-cell gel electrophoresis for detecting and identifying the impact of genotoxicants present in the air, water and, soil. Assessment of micronuclei in fishes, is one the most common, easy and, extensively used genotoxicity test to observe the genotoxic potential of environmental contaminants. Fishes are especially vulnerable to heavy metal pollution because they live and feed in their aquatic surroundings and are unable to avoid the harmful effects of pollutants. From this study, it has been found that $K_2Cr_2O_7$, a hexavalent chromium compound, is the most extensively used and erythrocytes are the most common cells studied for MN testing.

Key words: Chromium, Fish, Genotoxicity, Micronuclei.

1. INTRODUCTION

Environmental pollution has been an important public worry since the publication of book "Silent Spring" by Rachel Carson in 1962. Contamination of aquatic resources is a genuine issue. Despite the presence of significant enactment the contamination of the aquatic resources by harmful toxicants keeps on happening, with domestic and industrial effluents being the primary sources answerable for the pollution of aquatic resources [1-2]. Environmental pollutants such as heavy metals are hazardous to aquatic animals at physiological as well as genetic level, even if they are present in sub-lethal concentrations. In recent years, there has been an expanding biological and worldwide general wellbeing concern related with environmental pollution by heavy metals. Because of their bio-accumulative and non-biodegradable properties, their unreasonable defilement aquatic systems have evoked major ecological and wellbeing concerns around the world [3]. Human exposure to heavy metals has raised several folds as a result of an exponential increase of their use in several fields. Geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources are chief sources of heavy metals contamination in the environment [4]. Environmental contamination is exceptionally noticeable in point source regions such as mining, foundries and smelters, and other metal-based industrial activities.

Chromium is the 6th most bountiful naturally occurring heavy metal in the earth crust and considered as quite possibly the most widely recognized pervasive toxicant in the aquatic environment. In general, it is available in the environment in two valence states: Trivalent chromium and hexavalent chromium. The name of the metal is derived from the Greek word "chroma," which means color, because many of its compounds are intensely colored. The environmental behavior of Cr is largely a function of its oxidation state. Hexavalent Cr compounds are considered toxic to a variety of terrestrial and aquatic organisms. Hexavalent Cr compounds are strong oxidizers and highly soluble, while trivalent Cr compounds tend to form relatively inert precipitates at near-neutral pH [5]. Cytotoxicity of chromium has been reported by various authors in different studies along with bio-concentrating property of chromium. *In vitro,* cytological investigations have revealed that metal toxicity exerts influences on several cytological parameters including measurement of death of cell, viability, cellular morphology, cell metabolism, cell attachment or detachment, cell membrane permeability, proliferation, and growth kinetics [6-8].

In spite of the fact that, historically Cr(III) has truly been viewed as an essential trace element and early studies carried out during the 1950s by researchers' proposed a glucose tolerance factor (GTF) containing chromium as the dynamic part. However, further studies were failed to fully isolate chromium containing GTF [9,10]. The soluble hexavalent

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chromium is an environmental contaminant widely recognized to act as a carcinogen, mutagen and teratogen towards humans and animals. Contaminant of aquatic bodies by hexavalent chromium is a major issue and requires concerted attention because of its widely recognized nature of being a carcinogen, mutagen and teratogen toward humans and animals. Chromium was first suspected to be a human carcinogen in the late 19th century when epidemiological studies linked nasal tumors in Scottish chrome workers with chromium exposure [11]. In general, trivalent chromium compounds are more reactive than hexavalent with purified DNA and isolated nuclei; but hexavalent is well established mutagens and carcinogens having harmful effects on skin, respiratory tract and up to some extent on kidneys in humans [12].

Fishes are able to metabolize, concentrate, and store pollutants and can serve as a brilliant experimental animal for the study of mutagenic or carcinogenic potential of aquatic toxicants for environmental risk assessment. Tendency of fishes to bio-accumulate water toxicants especially heavy metals and consumption of these fishes by people makes the humans more susceptible and at greater risk to the chromium contamination. Genotoxic studies using cytogenetic endpoints as an indication of exposure to genotoxic substances in aquatic organisms such as fishes have been studied by a number of researchers [13-19]. MN test in fishes has been reported to be a very useful for genotoxicological studies for monitoring of natural water resources [14,20-22]. Peripheral blood micronucleus test (MNT) can serve as an early biological marker of exposure of fish to clastogenic pollutants in the aquatic environment.

2. MATERIALS AND METHODS

This review article provides an overview of the currently available research data on the genotoxic potential of a heavy metal, chromium. Attempts have been made to prepare an utmost consolidated manuscript on the topic. Data were collected from science journals of repute, published reports from international agencies and doctoral theses. Importance was given to the reproducible articles which are indexed in science journal database such as Scopus, Web of Science, Copernicus, and PubMed. Keywords were meticulously chosen and searched based on methodological scientific strategies. Keywords, for searching; were as follows: Chromium, fish, MN assay, genotoxicity, etc. Data related to fishes were chosen for the manuscript preparation. Scientific names of the experimental organisms were carefully screened before documentation. Experimental findings related to the genotoxicity of chromium of various researchers have been included at different parts of the present review to enhance the essence of the article.

3. VALENCE STATES OF CHROMIUM OF BIOLOGICAL AND ENVIRONMENTAL IMPORTANCE

As an element chromium is very stable, but is not usually found pure in nature. It has multiple oxidation states ranging from −2 to + 6, in which the trivalent Cr(III), and hexavalent Cr(VI) forms are the most common stable forms [23] and, Cr(II) is most unstable. There are a number of chromium compound of environmental importance (Table 1). The providence of chromium in the aquatic environment is reliant on its oxidation state. Cr(VI) first enters the cells, undergoes to metabolic reduction process, and changes to trivalent chromium. During this reduction process, there is formation of reactive oxygen species (ROS) which causes oxidative tissue damage and a cascade of cellular events. Cr(VI) is considered to be more toxic than Cr(III) because of its easy permeability through the cell membrane [24]. Hexavalent chromium has two main oxy-anionic forms CrO_4^{-2} and CrO_7^{-2} which are involved in reversible transformation [25].

$$2CrO_4^{-2} + 2H_3O^+ \leftrightarrow CrO_7^{-2} + 3H_2O$$

Table 1: Selected hexavalent chromium compounds

Chemical name	Chemical formula	CAS No.
Ammonium chromate	$(NH_4)_2CrO_4$	7788-98-9
Ammonium dichromate	$(NH_4)_2Cr_2O_7$	7789-09-5
Calcium chromate	$CaCrO_4$	13765-19-0
Chromium chloride	$CrCl_6$	14986-48-2
Chromium trioxide	CrO_3	1333-82-0
Potassium chromate	K_2CrO_4	7789-00-6
Potassium dichromate	$K_2Cr_2O_7$	7778-50-9
Sodium chromate	Na_2CrO_4	7775-11-3
Sodium dichromate	$Na_2Cr_2O_7$	10588-01-9
Chromium picolinate	$Cr(C_6H_4NO_2)_3$	14639-25-9
Chromium chloride	$CrCl_3$	10025-73-7

4. GENOTOXICITY

Genotoxicity can be defined as perturbation of the genome either by disruption of the DNA itself or by interference with its proper division and distribution in mitosis. MNT is one the extensively used genotoxicity assay used to asses genotoxic potential of environmental contaminants. It allows a convenient and easy application, particularly in genotoxicological studies with aquatic organisms. Initially, Howell [26] reported micronuclei in red blood cells and described it as tiny DNA-containing bodies present near the main nucleus of the cell. In early 1970's Matter and Schmid [27] and Heddle [28] have performed MN test to evaluate chromosomal damage in mammalian cells. Micronuclei are formed in all cell types after irregular division process. During the anaphase when a chromosome fragment or a whole chromosome is delayed with respect to the rest of chromosomes, it forms a small secondary nucleus called micronuclei [29]. In fishes MN test can be performed in different cell type, namely, erythrocytes, gill, kidney, hepatic, and fin cells. Due to large number and small size of chromosomes, and very low mitotic index only few species are suited for cytogenetic investigation [30]. The application of MN assay in fish hepatocytes has limitation due to low mitotic index of liver cells. In a study Yadav and Trivedi [31] evaluated the genotoxicity of CrO_3 in fish, *Channa punctata*, in terms of chromosomal aberrations and reported that acute exposure of Cr(VI) is genotoxic for *C. punctata*. Hexavalent chromium induced genotoxic effects in liver and kidney cells of goldfish, *Carassius auratus* exposed to various concentrations of Cr(VI) was observed by Velma and Tchounwou [32]. They observed DNA damage in both tissues in a concentration dependent manner using comet assay.

4.1. Chromium Interactions with DNA

Strong oxidizing property of Cr(VI) allows it to diffuse promptly in the tissues and can easily infiltrate cell membranes [33,34]. Its toxicity is a result of strong oxidizing action on cell membrane (phospholipid proteins) and nucleic acids [35]. However, its genotoxicity is a result of intracellular reduction of Cr(VI) to its most stable form Cr(III) with the generation of reactive intermediates such as Cr(IV) and Cr(V). During this reduction process, there is generation of ROS that can react with cellular DNA [36]. Usually trivalent form of chromium is found associated with DNA [37], which may form single strand breaks and DNA cross-links [38]. *In vivo* and *in vitro* studies carried out by several workers have revealed that Cr can damage DNA in several ways like it can induce single and double-strand breaks which may results in to CA, MN, sister chromatid exchange, alteration in DNA replication, and transcription [39-41].

4.2. Chromium Induced Micronuclei Formation in Fishes

Fishes can serve as great experimental animal to study the toxic and genotoxic potential of aquatic toxicants for environmental risk assessment [42] on account of their ability to quickly bio-concentrate and bio-accumulate waterborne toxicants. MN test is one of the excellent tools for both, *in vivo* and *in vitro* genotoxicity studies. A large number species of fishes, target tissue and variety of chromium compound have been used for genotoxicological evaluation in terms of micronuclei. Peripheral blood erythrocytes have been the most extensively used cells or target tissue to assess the genetic damage of aquatic pollutants in the fishes in terms of MN test (Table 2). The use of peripheral erythrocytes avoids the complex procedures associated with cell preparation.

Al-Sabti *et al*. [13] investigated the genotoxic effects of both trivalent and hexavalent chromium in erythrocytes of Prussian carp, *Carassius auratus gibelio,* and found considerably high frequency of micronuclei when fish were exposed to 10, 50, and 100 ng/ml of this metal. De Lemos *et al*. [43] also observed significantly increased frequency of MN in peripheral blood erythrocytes of the fathead minnow, *Pimephales promelas* exposed to sublethal concentration of Cr(VI) for various exposure periods. The induction was found to be decreased after 21 days of exposure. Zhu *et al*. [44] found that Carp, *Cyprinus carpio* treated with 0.001, 0.01, and 0.1 mg/L of Cr (VI) for different exposure periods showed significantly ($P < 0.01$) higher frequencies of micronuclei than those of control group. They also observed that MN frequencies increased after the first few days of exposure, peaked on day 9, and then smoothly changed. Significant increase in the frequency of micro nucleated erythrocytes and gills cells has also been reported by Cavas and Ergene-Gozukara [45] in *Oreochromis niloticus* when exposed to hexavalent chromium. Talapatra and Banerjee [46] found MN in the gill and kidney erythrocytes of *Labeo bata* fish raised in sewage-fed fish farms that contained heavy metal Chromium. They also reported a significantly ($P < 0.05$) increased level of micronuclei production in whole blood after exposing fish to sublethal quantities of potassium dichromate. Significantly increased levels of micronuclei in peripheral blood erythrocytes were also observed by Yadav and Trivedi (2009) [47] in fish *Channa punctata* exposed to $1/10^{th}$ of 96 h LC_{50} of CrO_3 after all exposure periods. They observed maximum frequency of micronuclei after 96 h of exposure period.

Rasal *et al*. [48] exposed fish, *Labeo rohita* to hexavalent chromium compound $K_2Cr_2O_7$ and reported that MN is a sensitive and rapid method to detect the effect of a genotoxic compound. Micronuclei and other nuclear abnormalities frequencies in peripheral blood of Nile tilapia (*Oreochromis niloticus*) treated with same chromium compound were also analyzed by Da Rocha *et al*. [49]. In comparison to a negative control, Chaudhary *et al*. [50] found a statistically significant increase in the frequency of MN in the peripheral blood erythrocytes of fish *Channa punctatus* using trivalent chromium compound. Kumar

Table 2: Chromium induced micronuclei in fishes

Fish species	2n	Target tissue	Chromium compound	Reference
Carassius auratus gibelio	100	Erythrocytes	$CrCl_3$, $K_2Cr_2O_7$	Al-Sabti *et al*. [13]
Pimephales promelas	50	Erythrocytes	$K_2Cr_2O_7$	De Lemos *et al*. [43]
Cyprinus carpio	100	Erythrocytes	$K_2Cr_2O_7$	Zhu *et al*. [44]
Oreochromis niloticus	44	Erythrocytes, Gill epithelial cells	Effluent of chromium processing plant	Cavas and Ergene-Gozukara [45]
Labeo bata	50	Erythrocytes from Gill and Kidney	Sewage containing Chromium	Talapatra and Banerjee [46]
Channa punctata	32	Erythrocytes	CrO_3	Yadav and Trivedi [47]
Labeo rohita	50	Erythrocytes	$K_2Cr_2O_7$	Rasal *et al*. [48]
Oreochromis niloticus	44	Erythrocytes	$K_2Cr_2O_7$	Da Rocha *et al*. [49]
Channa punctata	32	Erythrocytes	CrO_3	Chaudhary *et al*. [50]
Channa punctata	32	Erythrocytes, Gill epithelial cells	$K_2Cr_2O_7$	Kumar *et al*. [51]
Cyprinus carpio	100	Erythrocytes, Gill epithelial cells	$K_2Cr_2O_7$	Kumar *et al*. [52]
Catla catla	20	Erythrocytes	$Cr(NO_3)_3$	Arunachalam *et al*. [53]
Heteropneustes fossilis	56	Erythrocytes	$K_2Cr_2O_7$	Ahmed *et al*. [24]
Labeo dussumieri	50	Erythrocytes	$K_2Cr_2O_7$	Fernando *et al*. [54]
Cirrhinus mrigala	50	Erythrocytes	$K_2Cr_2O_7$	Mallesh *et al.* [55]
Labeo rohita	50	Erythrocytes, Gill epithelial cells	$K_2Cr_2O_7$	Nagpure *et al*. [56]
Oreochromis niloticus	44	Erythrocyte	$K_2Cr_2O_7$	Rocha *et al*. [57]
L. rohita and *H. fossilis*	50, 56	Erythrocyte	$K_2Cr_2O_7$	Bakshi [25]
Labeo calbasu, *Puntius sophore*, and *Mystus vittatus*	50, 58	Erythrocytes, Gill epithelial cells	Tannery effluents Containing Chromium	Nagpure *et al*. [58]
Channa punctatus	32	Erythrocytes	CrO_3	Prasad *et al*. [59]
Labeo rohita	50	Erythrocytes	$Cr(C_6H_4NO_2)_3$, $CrCl_3$	Asad *et al*. [60]
Ctenopharyngodon idellus	48	Erythrocytes	$K_2Cr_2O_7$	Handa and Jindal [61]
Channa punctatuss	32	Erythrocytes	CrO_3	Trivedi *et al*. [62,63]

et al. [51] investigated the genotoxicity of a hexavalent chromium compound ($K_2Cr_2O_7$) in a freshwater murrel fish, *Channa punctatus*, in an aquatic, static bio-system and found that Cr(VI) is a potential genotoxic compound and that the MNT is a sensitive and rapid method of detecting genetic effects. Kumar *et al*. [52] also investigated genotoxic potential of Cr(VI) in terms of MN induction in fish, *Cyprinus carpio* after exposure to the same chromium compound, potassium dichromate. Arunachalam *et al*. [53] used the MN test and Comet assay to assess chromium nitrate-induced acute toxicity in fingerlings of an Indian major carp, *Catla catla*. In the fish's peripheral erythrocytes, they noted a considerable increase in the frequency of micronuclei and bi-nuclei. Cr(VI) induced acute toxicity and genotoxicity in freshwater stinging catfish, *Heteropneustes fossilis* was evaluated by Ahmed *et al*. [28]. They exposed fish to sublethal concentrations (1/4th, 1/10th of 96 h- LC_{50} of $K_2Cr_2O_7$) and environmental concentration of Chromium reported in the river Buriganga for 48, 96, and 192 h. They conducted micronuclei test and found that frequency of micronuclei was increased after 48 and 96 h which decreased after 192 h of exposure. Genotoxicity of hexavalent chromium was evaluated in terms of micronuclei by Fernando *et al.* [54] in the fresh water fish *Labeo dussumeri* exposed to environmental levels. They also noticed a positive correlation between concentration and number of micronuclei. Mallesh *et al*. [55] subjected fingerlings of *Cirrhinus mrigala* to various doses of $K_2Cr_2O_7$, and evaluated the frequency of micronuclei in peripheral blood erythrocytes. Genotoxic potential of Cr(VI) in terms of micronuclei formation in epithelial gill cells and peripheral erythrocytes of freshwater fish *Labeo rohita* were reported by Nagpure *et al*. [56] when they exposed fish to the sublethal concentrations of potassium dichromate. The micronuclei test was performed in the adult freshwater fish Malabar Labeo (*Labeo dussumieri*) *in vivo* exposed to concentrations based on environmental levels (0.002–2.0 mg/L) and it was observed that the number of micronuclei in erythrocytes of fish increased significantly ($P < 0.05$) in comparison to control. Rocha *et al*. [57] investigated DNA damage and higher micronuclei frequencies in fish *Oreochromis niloticus* erythrocytes subjected to potassium dichromate.

In both, fish species *L. rohita* and *H. fossilis*, Bakshi [25] found a significant ($P < 0.05$) increase in micronuclei frequencies with increasing potassium dichromate concentrations compared to the control group. Nagpure *et al*. [58] also found significantly ($P < 0.05$) increased frequencies of micronuclei induction in erythrocytes and gill cells of fish *Labeo calbasu*, *Puntius sophore*, and *Mystus vittatus* obtained from polluted Ganga sites and subjected to tannery effluent. After exposure to sublethal concentrations of chromium trioxide, Prasad *et al*. [59] found a significant frequency of micronuclei induction in the erythrocytes of the freshwater fish *Channa punctatus*. Using the Comet assay, Asad *et al*. [60] investigated the effects of organic and inorganic chromium supplementation on growth performance and genotoxicity in *Labeo rohita*, and reported dose-related genetic damage by both organic [chromium picolinate - $Cr(C_6H_4NO_2)_3$] and inorganic (chromium chloride- $CrCl_3$) chromium in erythrocytes of fish. Handa and Jindal [61] observed eryptosis in *Ctenopharyngodon idellus* as a result of hexavalent chromium's genotoxic activity, which was apparent in the form of micronuclei. They subjected fish to sublethal concentrations of Cr(VI) (5.30 and 10.63 mg/L) for varying periods of time and concluded that hexavalent chromium produced eryptosis in *Ctenopharyngodon idellus*. Hexavalent chromium induced MN and amelioration potential of Withania sonmifera root extract in Channa punctatus has also been reported by Trivedi *et al*. [62]. In another study again Trivedi *et al*. [63] has reported genotoxic potential of hexavalent chromium in the same fish and also investigated decrease in micronuclei frequency when treated with *Rauwolfia serpentina*.

5. CONCLUSION

From the above review of literature, it has been found that micronuclei test in peripheral blood erythrocytes in fishes is most the most common and sensitive test in detecting genotoxicological potential of chromium compounds. Further, it has also been found that $K_2Cr_2O_7$ is the most extensively studied chromium compound. As fish fauna serves as a food source for humans, it is essential to know the impact of water pollution on these organisms. As fishes are at the top of the aquatic food chain and may directly affect the health of humans, this review would also be useful in providing an outline for the use of fishes and micronuclei test to scientific community and public officials involved in health risk assessment and management ensuring a better environmental condition for human health.

REFERENCES

1. L. D. Claxton, V. S. Houk, T. J. Hugles, (1998) Genotoxicity of industrial wastes and effluens, ***Mutation Research***, **410:** 237-243.
2. P. A. White, J. B. Rasmussen, (1998) The genotoxic hazards of domestic wastes in surface waters, ***Mutation Research***, **410:** 223-236.
3. S. S. Vutukuru, N. A. Prabhath, M. Raghavender, A. Yerramilli, (2007) Effect of arsenic and chromium on the serum amino-transferases activity in Indian major carp, *Labeo rohita*, ***International Journal of Environmental Research and Public Health***, **4**(**3**): 224-227.
4. Z. L. He, X. E. Yang, P. J. Stoffella, (2005) Trace elements in agro-ecosystems and impacts on the environment. ***Journal of Trace Elements in Medicine and Biology***, **19**(**2-3**): 125-140.
5. M. E. Losi, C. Amrhein, W. T. Frankenberger, (1994) Environmental biochemistry of chromium, ***Reviews of Environmental Contamination and Toxicology***, **136:** 91-121.
6. Z. Li, (2001) ***In vitro*** cytotoxicity of the organophosphorus pesticide parathion to FG9307 cells, ***Toxicology In Vitro***, **15**(**6**): 643-647.
7. K. Schirmer, D. G. Dixon, B. M. Greenberg, N. C. Bols, (1998) Ability of 16 priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill, ***Toxicology***, **127**(**1-3**):129-141.
8. H. Segner, (1998) Fish cell lines as a tool in aquatic toxicology, ***Fish Ecotoxicology***, **86:** 1-38.
9. F. Nielson, (2007) The clinical and nutritional importance of chromium still debated after 50 years of research. In: J. B. Vincent (Ed.), ***The Nutritional Biochemistry of Chromium*** (***III***), Amsterdam, Netherlands: Elsevier, Amsterdam, p265-276.
10. J. B. Vincent, D. Stallings, (2007) A history of chromium studies (1955-1995). In: J. B. Vincent (Ed.), ***The Nutritional Biochemistry of Chromium*** (III), Amsterdam: Elsevier, p1-40.
11. D. Newman, (1890) A case of adeno-carcinoma of the left inferior turbinated body and perforation of the nasal septum, in the person of a worker in chrome pigments, ***Glasgow Medical Journal***, **33:** 469-470.
12. S. Wang, X. Shi, (2001) Molecular mechanisms of metal toxicity and carcinogenesis, ***Molecular and Cellular Biochemistry***, **222:** 3-9.
13. K. Al-Sabti, M. Franko, B. Andrijanic, S. Knez, P. Stegnar, (1994) Chromium induced micronuclei in fish. ***Journal of Applied Toxicology***, **14:** 333-336.
14. K. Al-Sabti, C. D. Metcalfe, (1995) Fish micronuclei for assessing genotoxicity in water, ***Mutation Research***, **343:** 121-135.
15. K. K. Rishi, S. Grewal, (1995) Chromosome aberration test for the insecticide, dichlorvos, of fish chromosomes, ***Mutation***

***Research*, 344:** 1-4.
16. A. N. P. Mathew, S. Jahageerdar, (1999) Effect of heavy metal on the karyotype of *Channa punctatus*, ***Indian Journal of Fisheries*, 46(2)**: 167-172.
17. N. L. Maples, L. J. Bain, (2004) Trivalent chromium alters gene expression in the mummichog (*Fundulus heteroclitus*), ***Environmental Toxicology and Chemistry*, 23(3)**: 626-631.
18. K. K. Yadav, S. P. Trivedi, (2009a) Chromosomal aberrations in a fish, *Channa punctata* after *in vivo* exposure to three heavy metals, ***Mutation Research*, 678:** 7-12.
19. A. Bhatnagar, A. S. Yadav, N. Cheema, (2016) Genotoxic effects of chlorpyrifos in freshwater fish *Cirrhinus mrigala* using micronucleus assay, ***Advances in Biology***, 2016: 9276963.
20. B. Kushwaha, N. S. Nagpure, S. Srivastava, R. Kumar, M. S. Verma, (2003) Variation of micronuclei in peripheral blood cells of *Channa punctatus*, ***Indian Journal of Animal Sciences*, 73(10)**: 1192-1193.
21. D. Ali, N. S. Nagpure, S. Kumar, R. Kumar, B. Kushwaha, (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(10)**: 1823-1831.
22. K. K. Yadav, S. P. Trivedi, (2009b) Sublethal exposure of heavy metals induces micronuclei in fish, *Channa punctata*, ***Chemosphere*, 77:** 1495-1500.
23. K. Shekhawat, S. Chatterjee, B. Joshi, (2015) Chromium toxicity and its health hazards, ***International Journal of Advanced Research*, 3(7)**: 167-172.
24. M. K. Ahmed, G. K. Kundu, M. H. Al-Mamun, S. K. Sarkar, M. S. Khan, (2013) Chromium (VI) induced acute toxicity and genotoxicity in freshwater stinging catfish, *Heteropneustes fossilis*, ***Ecotoxicology and Environmental Safety*, 92:** 64-70.
25. A. Bakshi, (2016) ***Analysis of Anthropogenic Disturbances and Impact of Pollution on Fish Fauna of River Churni with Special Reference to Chromium Pollution* (*Doctoral Dissertation***), Kalyani University, Kalyani, India, p188.
26. W. H. Howell, (1891) The life history of the formed elements of the blood, especially of the red blood corpuscles, ***Journal of Morphology*, 4:** 57-116.
27. B. E. Matter, W. Schmid, (1971) Trenimon-induced chromosomal damage in bone marrow cells of six mammalian species, evaluated by the micronucleus test, ***Mutation Research*, 12:** 417-425.
28. J. A. Heddle, (1973) A rapid in vivo test for chromosomal damage, ***Mutation Research*, 18:** 187-190.
29. J. A. Heddle, M. C. Cimino, M. Hayashi, F. Romagna, M. D. Shelby, J. D. Tucker, P. Vanparys, MacGregor, (1991) Micronuclei as an index of cytogenetic damage: Past, present, and future. ***Environmental and Molecular Mutagenesis*, 18:** 277-291.
30. J. N. Cross, J. E. Hose, (1988) The reproductive cycle of demersal fishes in an area receiving urban wastes, abstract. In: ***6th International Ocean Disposal Symposium***, April 21-25, Pacific Grove, California, p116-117.
31. K. K. Yadav, S. P. Trivedi, (2006) Evaluation of genotoxic potential of chromium (VI) in *Channa punctata* fish in terms of chromosomal aberrations, ***Asian Pacific Journal Cancer Prevention*, 7:** 472-476.
32. V. Velma, P. B. Tchounwou, (2010) Chromium induced biochemical, genotoxic and histopathologic effects in liver and kidney of Goldfish, *Carassius auratus*, ***Mutation Research*, 698(1-2)**: 43-51.
33. M. Piscator, (1986) The dependence of toxic reactions on the chemical species of elements. In: M. Bernhard, F. E. Brinckman, P. J. Sadler (Eds.), ***The Importance of Chemical Speciation in Environmental Processes***, Berlin, Germany: Springer, Verlag, p59-70.
34. S. De Flora, K. E. Wetterhahn, (1989) Mechanism of chromium metabolism and genotoxicity, ***Life Chemistry Reports*, 7:** 169-244.
35. D. Chorvatovicova, Z. Kovacikova, J. Sandula, J. Navarova, (1992) Protective effect of sulphoethylglucan against hexavalent chromium, ***Mutation Research*, 302:** 207-11.
36. J. C. Mirsalis, C. M. Hamilton, K. G. O'Loughlin, D. J. Paustenbach, B. D. Kerger, S. Patierno, (1996) Chromium (VI) at plauaible drinking water concentrations is not genotoxic in the *in vivo* bone marrow micronucleus or liver unscheduled DNA synthesis assay, ***Environmental and Molecular Mutagenesis*, 28:** 60-63.
37. K. Hughes, M. E. Meek, R. Newhook, P. K. L. Chan, (1995) Speciation in health risk assessments of metals: Evaluation of effects associated forms present in the environment, ***Regulatory Toxicology and Pharmacology*, 22:** 213-20.
38. F. C. R. Manning, L. J. Blankenship, J. P. Wise, J. Xu, L. C. Bridgewater, S. R. Patierno, (1994) Induction of inter nucleosomal DNA fragmentation by carcinogenic chromate: Relationship to DNA damage, genotoxicity and inhibition of macromolecular synthesis, ***Environmental Health Perspective*, 102:** 159-167.
39. A. Zhitkovich, V. Voitkun, M. Costa, (1996) Formation of the amino acid-DNA complexes by hexavalent and trivalent chromium *in vitro*: Importance of trivalent chromium and the phosphate group, ***Biochemistry*, 35:** 7275-7282.
40. T. O'Brien, J. Xu, S. R. Patierno, (2001) Effects of glutathione on chromium-induced DNA crosslinking and DNA polymerase arrest, ***Molecular and Cellular Biochemistry*, 222:** 173-182.
41. S. T. Matsumoto, M. A. Marin-Morales, (2004) Mutagenic potencial of the water of a river that receives tannery effluent using the *Allium cepa* test system, ***Cytologia*, 69:** 399-408.
42. K. Belpaeme, K. Delbeke, L. Zhu, M. Kirsch-Volders, (1996) Cytogenic studies of PCB77 on brown trout (*Salmo trutta fario*) using the micronucleus test and the alkaline comet assay, ***Mutagenesis*, 11(5)**: 485-492.
43. C. T. De Lemos, P. M. Rodel, N. R. Terra, B. Erdtmann, (2001) Evaluation of basal micronucleus frequency and hexavalent chromium effects in fish erythrocytes, ***Environmental Toxicology and Chemistry*, 20(6)**: 1320-1324.
44. Y. Zhu, J. Wang, Y. Bai, R. Zhang, (2004) Cadmium, chromium, and copper induce polychromatocyte micronuclei in Carp (*Cyprinus carpio* L.), ***Bulletin of Environmental Contamination and Toxicology*, 72(1)**: 78-86.
45. T. Cavas, S. Ergene-Gozukara, (2005) Induction of micronuclei and nuclear abnormalities in *Oreochromis niloticus* following exposure to petroleum refinery and chromium processing plant effluents, ***Aquatic Toxicology*, 74:** 264-271.
46. S. N. Talapatra, S. K. Banerjee, (2007) Detection of micronucleus and abnormal nucleus in erythrocytes from the gill and kidney of *Labeo bata* cultivated in sewage-fed fish farms, ***Food Chemical Toxicology*, 45(2)**: 210-215.
47. K. K. Yadav, S. P. Trivedi, (2009) Micronucleus assay in a fish model after *in vivo* exposure to chromium (VI). In: B. N. Pandey, (Ed.), ***Cell and Molecular Biology***, New Delhi: APH Publishing Corporation, p1-14.

48. K. Rasal, A. Rasal, N. Makwana, (2011) Micronucleus test as a cytogenetic marker for evaluation of genotoxicity in fish, *Labeo rohita*, ***The Asian Journal of Animal Science***, **6**(**1**): 32-34.
49. C. A. M. Da Rocha, C. D. F. Gomes, R. F. G. Jr. Riberio, R. H. D. Pinheiroet, (2011) Detection of micronuclei and other nuclear abnormalities in *Oreochromis niloticus* exposed to potassium dichromate, ***Global Veterinaria***, **7**(**3**): 301-304.
50. J. Choudhary, Abha, A. M. J. (2012) Cytogenetic effect of chromium trioxide in an air breathing teleost *Channa punctatus* (Bloch), ***Biological Science***, **2**(**1**): 246-253.
51. P. Kumar, R. Kumar, N. S. Nagpure, P. Nautiyal, A. Dabas, B. Kushwaha, W. S. Lakra, (2012) Genotoxic and mutagenic assessment of hexavalent chromium in fish following *in vivo* chronic exposure, ***Human and Ecological Risk Assessment***, **18:** 855-870.
52. P. Kumar, R. Kumar, N. S. Nagpure, P. Nautiyal, B. Kushwaha, A. Dabas, (2013) Genotoxicity and antioxidant enzyme activity induced by hexavalent chromium in *Cyprinus carpio* after *in vivo* exposure, ***Drug and Chemical Toxicology***, **36**(**4**): 451-460.
53. K. D. Arunachalam, S. K. Annamalai, J. K. Kuruva, (2013) Chromium induced DNA damage by alkaline comet assay and oxidative stress in *Catla catla*, ***American Journal of Environmental Science***, **9**(**6**): 470-482.
54. V. A. K. Fernando, C. D. Dangalle, I. C. Perera, J. Weerasena, (2015) Genotoxic Effects of Hexavalent Chromium on *Labeo dussumieri*, a Food Fish in Sri Lanka, ***4th Global Summit on Toxicology***, August 24-26.
55. B. Mallesh, P. Pandey, K. Kumar, A. Vennila, S. Shukla, R. P. Raman, S. Kumar, (2015) Bioconcentration of hexavalent chromium in different organs and induction of micronuclei in peripheral blood cells of *Cirrhinus mrigala* (Ham, 1822), ***Indian Journal of Animal Sciences***, **85**(**5**): 92-100.
56. N. S. Nagpure, R. Srivastava, R. Kumar, B. Kushwaha, S. K. Srivastava, P. Kumar, A. Dabas, (2015) Assessment of genotoxic and mutagenic potential of hexavalent chromium in the freshwater fish *Labeo rohita* (Hamilton, 1822), ***Drug and Chemical Toxicology***, **38**(**1**): 9-15.
57. C. Rocha, P. Cardoso, L. Cunha, C. Gomes, R. R. Junior, R. H. Pinheiro, M. H. Costa, R. Burbano, (2015) Mutagenic effects of potassium dichromate as evaluated by means of animal and plant bioindicators, ***In Vivo***, **29**(**6**): 729-736.
58. N. S. Nagpure, R. Srivastava, R. Kumar, A. Dabas, B. Kushwaha, P. Kumar, (2017) Mutagenic, genotoxic and bioaccumulative potentials of tannery effluents in freshwater fishes of River Ganga, ***Human and Ecological Risk Assessment: An International Journal***, **23:** 98-111.
59. R. Prasad, M. Kumar, S. P. Trivedi, (2017) Antigenotoxic effect of turmeric powder extract curcumin against chromium trioxide induced genotoxicity in fish *Channa punctatus*, ***Journal of Entomology and Zoology Studies***, **5**(**1**): 89-94.
60. F. Asad, M. S. Mubarik, T. Ali, M. K. Zahoor, R. Ashrad, S. Qamer, (2019) Effect of organic and in-organic chromium supplementation on growth performance and genotoxicity of *Labeo rohita*, ***Saudi Journal of Biological Sciences***, **26:** 1140-1145.
61. K. Handa, R. Jindal, (2020) Genotoxicity induced by hexavalent chromium leading to eryptosis in *Ctenopharyngodon idellus*, ***Chemosphere***, **247:** 125967.
62. S. P. Trivedi, R. Prasad, A. A. Khan, (2020) Amelioration potential of *Withania sonmifera* root extract on hexavalent chromium induced micronucleus in *Channa punctatus* (Bloch, 1793), ***Journal of Environmental Biology***, **41**(**4**): 672-679.
63. S. P. Trivedi, V. Kumar, S. Singh, M. Kumar, (2021) Efficacy evaluation of *Rauwolfia serpentina* against chromium (VI) toxicity in fish, *Channa punctatus*, ***Journal of Environmental Biology***, **42**(**3**): 659-667.

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