Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 05, pp.30245-30251, May, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ASSESSMENT OF ENVIRONMENTAL STRESS BY THE MICRONUCLEUS TEST ON CYPRINID FISH AS A MODEL FOR *IN SITU* DETECTION OF MUTAGENS IN FRESHWATER

[1]Maryum Meraj, [1,*]Md. Niamat Ali, [1]Bashir A. Ganai, [2]F. A. Bhat and [1]Hilal A. Ganaie

[1]Cytogenetics and Molecular Biology Research Laboratory, Centre of Research for Development (CORD), University of Kashmir, Srinagar-190006, J & K, India
[2]Division of Fisheries, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir (SKUAST-K), J & K, India

ARTICLE INFO

Article History:
Received 18th February, 2016
Received in revised form
06th March, 2016
Accepted 22nd April, 2016
Published online 10th May, 2016

Key words:

Fish,
Micronucleus test,
SEM,
Environmental stress,
Dal Lake,
Mansbal Lake.

ABSTRACT

Changes in micronuclei frequency of fish captured from stressed environments may represent a reliable tool in revealing sub lethal effects of the pollutants found in aquatic ecosystems. The response patterns of peripheral erythrocyte micronuclei were assessed in fish caught at Dal Lake and Mansbal Lake which have different pollution levels with the aim to evaluate the suitability of the MN, for the Halics detection of mutagens in freshwaters. As indicator species, Cyprinid fish (*Cyprinus carpio* and *Carassius carassius* L.) were used because of their ecological significance. Blood samplings were performed on fish immediately after capture and repeated at different time intervals (15, 30, 45 and 60 days) on the same fish individuals after transfer to clean environment (aquariums) in the laboratory. The MN formation in the peripheral erythrocytes, authenticated by scanning electron microscopy (SEM), was found significantly higher ($p < 0.01$) in both species of fish caught from Dal Lake in comparison to fish from Mansbal Lake and also from the positive control (EMS, 5mg/L). Cyprinid fish examined after 15, 30, 45 and 60 days of maintenance after capture presented a remarkable decrease resulting in recovery up in MN frequency in comparison with the frequency observed at capture. Results suggested the suitability of the test species used as tools in environmental monitoring programs of risk assessment in fresh water environments.

Citation: Maryum Meraj, Md. Niamat Ali, Bashir A. Ganai, Bhat, F. A. and Hilal A. Ganaie, 2016. "Assessment of Environmental Stress by the Micronucleus test on Cyprinid Fish as a Model for in Situ Detection of Mutagens in Freshwater", *International Journal of Current Research*, 8, (04), 30245-30251.

INTRODUCTION

Aquatic ecosystems are major recipients of pollutants which over time can have serious consequences for biota that may not become apparent until changes occur at the population or ecosystem level, a point at which it may be too late to take effective counter measures. The need to detect and assess the impact of pollutants, particularly at low, sub lethal concentrations, on environmental quality has led to development of a range of biological responses measured in number of different species (Linde-Arias *et al*., 2008). Studies reveal the fact that a number of chemicals contaminating the environment have carcinogenic or mutagenic effects. The major sources for the mutagenic and carcinogenic substances are industrial and agricultural activities (Bogoni *et al*., 2014). Xenobiotics from these sources ultimately contact the aquatic ecosystems. Although many hazardous substances exist in water and sediment and they are accumulated by aquatic organisms and trigger DNA or cellular damage and even affects the ecosystem by passing through the tropic chain (Izquierdo *et al*., 2003). In recent years several studies have evaluated the impact of agricultural and industrial effluents on river waters using different assays (Lemos and Erdtmann, 2000; Vargas *et al*., 2001; Vigano *et al*., 2002; Tagliari *et al*., 2004; Ohe *et al*., 2004; Ergene *et al*., 2007 and Lemos *et al*., 2007). Anthropogenic activities as sources of increased toxic substance content in aquatic systems are now common in Kashmir. In addition to classical studies of freshwater pollution based on media physicochemical analyses, efforts are being made to identify and design new approaches and tools for diagnosing water toxicity. Genotoxicity of contaminated waters has been studied well using standard *in vitro* genotoxicity tests (Arslan *et al*., 2015). Studying DNA damage at the level of chromosome constitute a necessary part of genetic toxicology (Fenech, 2000) because chromosomal

****Corresponding author: Md. Niamat Ali,***
Cytogenetics and Molecular Biology Research Laboratory,
Centre of Research for Development (CORD), University of Kashmir, Srinagar-190006, J & K, India.

mutation plays the most important role in cancer formation. Thus, biomarkers have been intensively used in research programs to assess the pollution of the water bodies (Bolognesi and Fenech, 2012; Arslan *et al*., 2015). The significant deviation in the effect of a biomarker compared to its basal or reference value provides additional information regarding the extent of the impacts caused by stressful environmental agents. In the case of fish, foreign chemicals present in their environment may act as stressors threatening or disturbing the organism; in that case, animal homeostasis initiates an integrated response, i.e. a set of compensating and adaptive responses that readjust metabolic processes in order to cope with the effects of pollutants. Compensating for the effects of chemical stressors causes reallocation of metabolic energy away from investment activities and towards the restoration of homeostasis. The restoration of the damaged homeostasis in a stressed fish is associated with an increase in metabolic rate in relation to the non-stressed condition (Beyers *et al*., 1999). In addition, research into the recovery of altered biomarkers back to original levels after transfer to clean media provides information in connection with the reversibility of the detected changes if adequate remediation measures are applied. This information is also useful in the prediction of the capacity of a particular population to survive in disturbed environments. The use of fish as bio-indicators of pollutant effects is being more and more used since fishes are very sensitive to changes in their environment and play significant roles in assessing potential risk associated with contamination in aquatic environment (Dar *et al*., 2015; Nwani *et al*., 2010); they are frequently used test organisms for studying cytotoxicity, water toxicity and genotoxicity. Among the many mutagenesis assays, micronuclei (MN) test is one of the most simple, sensitive and reliable techniques used to determine genetic changes in the organisms; furthermore it is not strongly dependent on any karyotypic characteristic. For all these reasons, the micronucleus test in fish erythrocyte seems to be a promising test in the investigation of environmental mutagenesis (Al-sabti and Metcalfe, 1995).

As indicator species, *Cyprinus carpio* and *Carassius carassius* was chosen because of their ecology, wide distribution in fresh water environment of Kashmir, availability throughout the seasons, easy acclimatization in the laboratory conditions and commercial importance make these species as an excellent test specimen for geno-toxicological studies. These considerations have prompted interest in the development of such techniques and its use as bioindicators for monitoring the genomic damage from environmentally hazardous contaminants in the aquatic environment. In the present study, micronucleus frequency in fish erythrocytes has been evaluated in *Cyprinus carpio* and *Carassius carassius* from two fresh water environments Dal Lake and Mansbal Lake characterized by different pollution levels, and their recovery once animals were transferred to unpolluted environment as a consequence of an activation of compensatory responses.

MATERIALS AND METHODS

Experimental fish specimens

Two Cyprinids, *Cyprinus carpio* and *Carassius carassius* L. (Family: Cyprinidae and Order: Cypriniformes), were selected as model organism. The average length (cm) and wet weight (g) (± SD) of *Cyprinus carpio* were recorded as 18.12 ± 0.62 cm and 77 ± 6.782 g, for Dal Lake and 17.62 ± 1.70 cm and 66.25 ± 17.97 g, for Mansbal Lake respectively and for *Carassius carassius* were as 15 ± 1.82 cm and 46.13 ± 8.16 g, for Dal Lake and 15.18 ± 0.831 cm and 39.86 ± 3.44 g, for Mansbal Lake respectively. Specimens were captured with the help of a local fisherman, from the two different sites, Dal Lake (34°07′N 74°52′E), and Mansbal Lake (34°15′ N, 74°40′E). The Dal Lake is an urban lake that lies to the east of Srinagar city, at the foot of Zabarwan Hills, and is situated at an average elevation of 1,583 m (5,194 ft) above sea level with a maximum depth of 6 m (20 ft). Mansbal Lake,a rural lake situated at a distance of 32 km from Srinagar city with length and breadth of 3.2 and 1 km, respectively. The lake is situated at the altitudinal zone of 1,585-1,600 m asl with a maximum depth of 13 m. Recent studies by Zargar *et al*., 2012 have attested that the Dal Lake have reached to the level of eutrophic condition, but the level of trophic state varies, with Dal Lake being the most eutrophic and Mansbal Lake being the least nutrient enriched.

The fish captured were assigned into two groups:

- Specimens used immediately to determine Micronuclei. These were placed in glass jars with ice-cold tap-water and transported on ice within a period no longer than 3h and
- Specimens placed in containers and transported to the recovery sites,

Recovery test in the laboratory

The fish captured from both the sites were transported to the laboratory and were subjected to a prophylactic treatment by bathing in a 0.05% aqueous solution of potassium permanganate for two min to avoid any fungal or dermal infection. The fish were then maintained in well aerated 60 L glass aquariums and properly acclimatized for 60 days under controlled and constant environmental conditions at 19.7 ± 2.6 C, continuous airing with 24h aged in decholrinated tap water (pH 7.2 - 8.4) and fed ad libitum with commercially available fish food (Feed Royal^{R}, Maa Agro foods, Visakhapatnam, Andhra Pradesh, India) five times a week. Water was changed on alternative days. The metabolic waste products were siphoned off daily to prevent increase of ammonia in water and no fish mortality occurred during this period. Every effort, as suggested by Bennett and Dooley (1982), was made to maintain optimal conditions during acclimatization.

In vivo experiment

The experiment was carried out in total 40 aquaria, containing 60 L dechlorinated and well aerated tap water with 5 fish in each aquarium. The positive control group was maintained by exposing *C. carpio* and *C. carassius* to Ethyl methanesulfonate [(CAS no. 62-50-0), 5mg/L, concentration selection based on previous investigation; Cavas and Konen, 2008, Cavas, 2011]. The experiment was conducted over a period of 60 days each and the autopsy was done at the time of capture, after 15, 30, 45 and 60 days of maintenance.

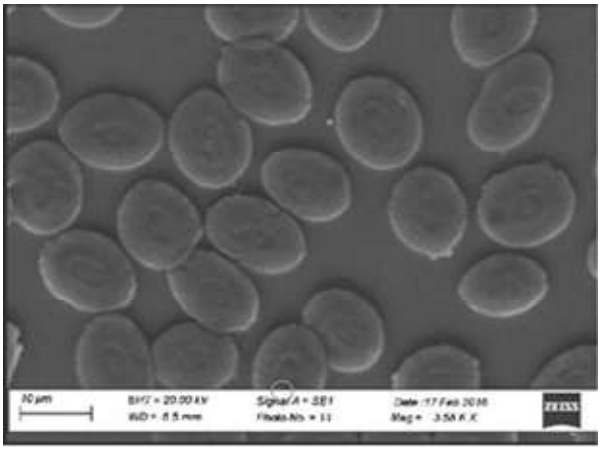

Fig. 1a

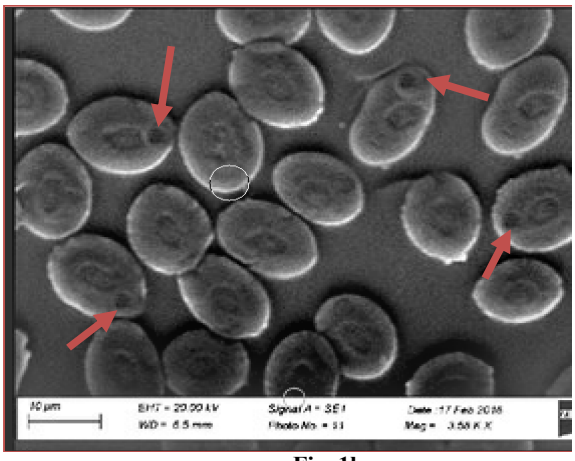

Fig. 1b

Fig. 1. Scanning electron microscopic images of 1a) Normal erythrocytes; 1b) Micronucleus in erythrocytes (arrow) of *Cyprinus carpio* and *Carassius carassius* (1000x) =10µm)

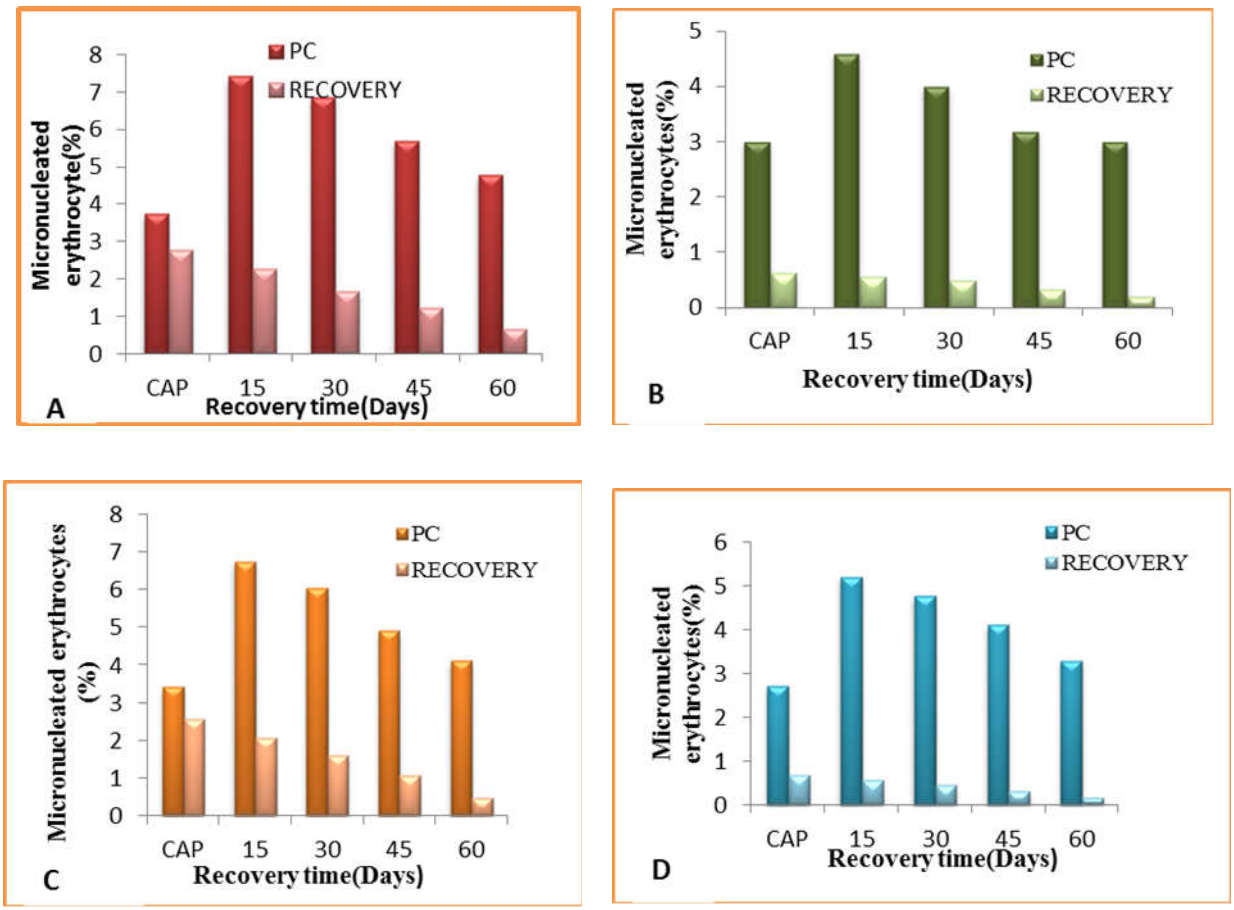

Fig. 2. Micronucleus frequencies in: (A) *Cyprinus carpio* from Dal lake (positive control and recovery time); (B) *Cyprinus carpio* from Mansbal lake (positive control and recovery time). In both cases fish were tested for MN at capture, after 15, 30, 45 and 60 days of maintenance in aquariums., and (C) *Carassius carassius* from Dal lake (positive control and recovery time); (D) *Carassius carassius* from Mansbal lake (positive control and recovery time)

On each sampling interval, micronucleus assay were carried out from the blood of five fish as per standard protocols. Some important physico-chemical characteristics of water like temperature, pH, dissolved oxygen and conductivity of aquarium water were analyzed throughout the study by standard methods (APHA, AWWA and WPCF, 2005).

Measurements were made using the following equipment/ method (s): water temperature, Celsius mercury thermometer calibrated up to 0.1°C, digital pH meter (Microprocessor pH System-1011E); conductivity, Systronics model 104 conductivity meter and dissolved oxygen with the Azide modification of Winkler method (APHA, 2005).

Genotoxicity biomarker

Micronucleus test

Prior to blood collection, fish were anaesthetized with 0.12 g L^{-1} benzocaine (Dar *et al*., 2015). Peripheral blood samples were withdrawn by severing caudal peduncle. Immediately after blood collection, a thin smear of blood was made by applying two drops of blood on pre-cleaned, grease free frosted slides, two for each specimen, using the standard fish micronucleated erythrocytes method of Al-Sabti and Metcalfe (1995). The smeared slides were air-dried for at least 2-3 hrs to upto overnight in a dust and moisture free environment at room temperature. Next day, the slides were fixed by dipping in cold absolute methanol (4 C) for 15 min and again left to air dry at room temperature for 1 h. Finally, the slides were stained in May-Grunwald stain for 5-10 min followed with 6% Giemsa in phosphate buffer for 30 min. The slides were then washed thoroughly in double distilled water, dried, dehydrated (30. 50, 70, 90% and absolute ethanol), cleared in xylene and made permanent with DPX-mounting.

Micronuclei (MN) scoring

Slides were selected on the basis of staining quality, then coded, randomized and scored blindly. The criteria for identifying MN were according to (Fenech *et al*., 2003). While identifying MN every care was taken. Only non-refractile objects having same staining intensity as principal nucleus and with dark blue colour and smooth edges were counted.For each sampling time, five fish were used and replicate slides per fish were prepared. About 1000-1200 cells were examined from each slide, i.e., a minimum of 10,000 erythrocytes were scored in each group for the presence of MN. The frequency of MN/fish was calculated per 1000 cells (Dar *et al*., 2015) and was evaluated by scoring the slides under oil immersion at 1000x magnification using Olympus BX 50 microscope (Tokyo, Japan).

Scanning electron microscope (SEM)

For more authentications of MN results, SE images were deduced from the higher resolution efficient SEM technique. The SEM was carried out by standard procedure (Reimer, 1985; Dar *et al*., 2015). Briefly, the aforementioned micro nucleated slides were reshaped, sputter-coated with platinum to a layer of 3–5 nm, and exclusively examined in the secondary electron mode, at an accelerating voltage of 10 kV, with a scanning electron microscope (JSM6510LV, JEOL, Japan).The images were recorded simultaneously with Digiscan™ hardware and processed with Digital Micrograph 3.4.4 software (Gatan, Inc., Pleasantdon, CA, USA).

Statistical Analysis

Statistical analysis of data to verify the significant difference in the incidence of micronucleus between samples; and between positive control and recovery time (days) at 0.05 and 0.01 level of significance was performed by Mann-Whitney U test to analyze the frequency of micronuclei. All the statistical calculations were performed with the SPSS (version 16.0) computer program (SPSS Inc. Chicago, IL, USA).

RESULTS

Physicochemical Properties of the Aquarium Water

The aquarium water temperature varied from 18.4–22.3 °C and the pH ranged from 7.2–8.4. The dissolved oxygen concentration was normal, ranged from 7.6–8.2 mg/l, total alkalinity 68–74 mg/l and the conductivity of the water ranged from 211 to 239 µM/cm during experimental period.

Micronucleus as Genotoxicity Biomarker: No mortality was observed throughout the experiments .The result of MN analysis in erythrocytes of *C. carpio* and *C. carassius* in each one of the tests are summarized in table 1 and 2.

Table 1. Mean micronuclei frequency (%) in peripheral erythrocytes of *C. carpio* collected at Dal and Mansbal Lake (n = 10000 cells/recovery time)

Site		Recovery time (Days)				
		Capture	15	30	45	60
Dal Lake	PC	3.74 ± 0.250^{a2}	7.42 ± 0.168^{b2}	6.88 ± 0.251^{b2}	5.7 ± 0.298^{b2}	4.78 ± 0.290^{b1}
	Recovery	2.78 ± 0.261^{B2a}	2.28 ± 0.226^{Bb2}	1.66 ± 0.092^{Bb2}	1.22 ± 0.142^{Bb2}	0.66 ± 0.074^{Bb2}
Mansbal Lake	PC	3.0 ± 0.353^{b}	4.6 ± 0.430^{b}	4 ± 0.316^{b}	3.2 ± 0.489^{b}	3 ± 0.418^{b}
	Recovery	0.62 ± 0.05^{B2b}	0.56 ± 0.05^{Bb}	0.48 ± 0.073^{Bb}	0.32 ± 0.037^{Bb2}	0.2 ± 0.031^{Bb2}

PC: positive control (EMS), Values with different capital letter superscripts ($^{A}P < 0.05$: Significant, $^{B}P < 0.01$: highly significant, differ significantly between the sites, whereas values with different numeric superscripts ($^{1}P < 0.05$: Significant, $^{2}P < 0.01$: highly significant) differ significantly between recovery time; and values with different small letter superscripts ($^{a}P < 0.05$: Significant, $^{b}P < 0.01$: highly significant) differ significantly between positive control and recovery times.

Table 2. Mean micronuclei frequency (%) in peripheral erythrocytes of *C. carassius* collected at Dal and Mansbal Lake (n = 10000 cells/recovery time)

Site		Recovery time (Days)				
		Capture	15	30	45	60
Dal Lake	PC	3.42 ± 0.177^{a2}	6.76 ± 0.250^{b2}	6.04 ± 0.136^{b2}	4.92 ± 0.131^{b2}	4.12 ± 0.135^{b1}
	Recovery	2.58 ± 0.190^{B2a}	2.08 ± 0.208^{Bb}	1.62 ± 0.124^{Bb}	1.06 ± 0.06^{Bb2}	0.48 ± 0.086^{Ab2}
Mansbal Lake	PC	2.72 ± 0.193^{b2}	5.22 ± 0.222^{b}	4.78 ± 0.224^{b}	4.12 ± 0.196^{b}	3.3 ± 0.20^{2b}
	Recovery	0.68 ± 0.086^{B2b}	0.56 ± 0.024^{Bb}	0.46 ± 0.092^{Bb}	0.32 ± 0.037^{Bb}	0.16 ± 0.024^{Ab2}

PC: positive control (EMS), Values with different capital letter superscripts ($^{A}P < 0.05$: Significant, $^{B}P < 0.01$: highly significant) differ significantly between the sites, whereas values with different numeric superscripts ($^{1}P < 0.05$: Significant, $^{2}P < 0.01$: highly significant) differ significantly between recovery time; and values with different small letter superscripts ($^{a}P < 0.05$: Significant, $^{b}P < 0.01$: highly significant) differ significantly between positive control and recovery times.

There were statistically high significant difference ($p < 0.01$) in the MN frequencies of both the species between the Dal fish tested immediately after capture and those from the Mansbal; and between the fish caught from Dal lake at the time of capture and after 60 days of maintenance. Significant differences ($p < 0.05$) in MN frequencies of both species were observed between the control group (positive control) and samples from Dal Lake, immediately after capture (Fig 2: A, C). After transferable to clean media (recovery in aquariums) observations on the same fish after 15, 30, 45 and 60 days of maintenance showed clearly high significance ($p < 0.01$) towards recovery up resulting in decrease in MN frequencies (Fig 2: A and C). Both the species from Mansbal Lake showed immediately after capture, a significant difference ($p < 0.01$) in MN frequencies compared to the control group. Noticeable decreases in MN frequency were observed in both of the species after maintenance in clean media (Fig 2: B and D). The MN values (15, 30, 45 and 60 days) were significantly ($p < 0.01$) lower than the value observed at capture. Micronuclei frequencies in both species from Dal Lake are significantly higher ($p < 0.01$) than Mansbal Lake. The results of MN were more authenticated by SEM analyses as shown in Fig.1

DISSCUSION

On the basis of previous research carried out by different limnologists on the Dal and Mansbal Lake, Kashmir, displayed different environmental stress. Recent studies by Zargar *et al*., 2012 and Balkhi and Yousuf, 1992, 1996, have attested that the Dal Lake is highly eutrophic and Mansbal Lake being the least nutrient enriched. As the valley is devoid of chemical factories nutrient input from domestic sewage define the main source of pollution and the indiscriminate use of pesticides in and around by vegetable growers on floating gardens of Dal Lake. The marked difference in the water quality of the two lakes clearly depicts the influence of anthropogenic stresses on the lakes. These results are also in confirmation with the loading concept of Rawson (1939), Ohle (1956), Edmondson (1961) and support the findings of Pandit and Yousuf (2002). Genotoxicity as an important biomarker is useful for assessing the effects of chemicals in aquatic ecosystems, especially chemicals that are capable of affecting the health and demography of different aquatic organisms (Zapata *et al*., 2016**).**

The contamination of aquatic environments poses serious consequences for the welfare of the organisms exposed because pollutants may induce mutations and cancer (Beyersmann and Hartwig, 2008) as well as cell death (Tan *et al*., 2008). The presence of micronuclei represents a parameter for determining the extent of damage caused by an environmental agent to the process of cell division of the affected tissue (Vine, 1990) and reveals threats that cannot be detected through chemical or physical analyses. Fish serve as sentinel organism for ecotoxicological studies because they play a number of roles in tropic web, accumulate toxic substances and respond to low concentration of mutagens in a similar way to higher vertebrates (Dar *et al*., 2014). Therefore, the use of fish biomarkers as indices of the effects of pollution, are of increasing importance and can permit early detection of aquatic environmental problems (Nwani *et al*., 2010). Different studies showed that MN can be affected by many factors such as age, sickness, species, feeding, chemical and physical agents (Al-Sabti and Metcalfe, 1995; Saleh and Zeytinoglu, 2001). So to eliminate these factors, healthy, young and active individual had been chosen from the same species, *C. carpio* and *C. carassius*. Therefore, the differences detected in the MN frequencies cannot be attributed to the effect of these parameters. The results of the MN test carried out in this study on *C. carpio* and *C. carassius* raised several points taken into considerations. The most remarkable result is that MN frequency appears to be strongly related to the water quality of the different environments examined. The relationship between MN frequency and pollution levels observed in fresh water fish species reflects what already observed by different authors in marine environment (Hose *et al.,*1987; Minissi *et al*., 1996; Arslan *et al*., 2015). The presence of different pesticide residues and pollutants in Dal Lake was previously evidenced among the human population living within Dal Lake and also from the fish, *Schizothorax niger* (Banday *et al*., 2012; Zargar *et al*., 2012; CSIR-NEERI, 2013). On the contrary, the low MN frequencies observed in the Mansbal Lake lead to the conclusion that in this lake genotoxic agents are not detectable. Noticeable decreases in MN frequency were observed in both of the species after transfer to clean environment. The MN values (15, 30, 45 and 60 days) were significantly lower than the value observed at capture. Same was reported by Minissi *et al*. (1996) in erythrocytes of *Barbus plebegus,* examined at different time intervals after maintenance in the laboratory.

The improvement in MN frequency of the test fish species when transferred to pollutant-free freshwater suggests that pollutants entering into the system are slowly eliminated and hence the fish shows recovery from the pollutant toxicity. The chance to follow the MN frequency with repeated blood samplings on the same individuals at capture and different time intervals after maintenance in laboratory conditions demonstrated a recovery capacity after removal from natural environment, hence can be concluded that the MN test in fish is indicative of short term cytogenetic damage. The results of the present investigation on the genotoxic potential of the polluted water suggested a serious concern about its potential danger to aquatic organisms, especially to fish, and indirectly to human beings. Moreover, in the absence of other convenient or practical methods, the MN will continue to play an important role in assessing the genotoxicity induced by different pollutants. MN frequencies appear to be the most ductile although after suffering significant modifications they recover to their minimal values when the animals are kept in clean environment. In connection with the above, *C.carpio* and *C.carassius* are confirmed as being suitable species for use in integrated biomonetiring of freshwater toxicity. However, further studies are needed to explore the biological consequences of DNA damage in aquatic organisms due to deleterious effects of polluted water of Dal Lake and to formulate future strategies for safeguarding aquatic organisms and environment.

Acknowledgment

The authors are grateful to the Director of Centre of Research for Development (CORD), University Kashmir, India for

providing the entire necessary facilities. The first author is also thankful to Prof. T.C. Nag, Incharge Electron Microscopy Facility, All India Institute of Medical Sciences (AIIMS), New Delhi for providing the SEM facility.

REFERENCES

Al-Sabti, K. and Metcalfe, C.D. 1995. Fish micronuclei for assessing genotoxicity in water. *Mutation Research*, 343: 121-135.

Ansari, R. A., Rahman, S., Kaur, M., Anjum, S. and Raisuddin, S. 2011. In vivo cytogenetic and oxidative stress-inducing effects of cypermethrin in freshwater fish, *Channa punctata* Bloch. *Ecotoxicology and Environmental Safety*, 74:150–6.

Arkhipcuk, V.V. and Garanko, N.N. 2005. Using the nucleolar biomarker and the micronucleus test on in vivo fish fin cells. *Ecotoxicology Environmental Safety*, 62: 42–52.

Arslan, O.C., Boyacioglu, M., Parlak, H., Katalay, S. and Karaaslan, M.A. 2015. Assessment of micronuclei induction in peripheral blood and gill cells of some fish species from Aliag˘a Bay Turkey. *Marine Pollution Bulletin*, 94: 48–54.

AWWA., APHA. and WPCF. 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Publication of Health Association, Washington, DC.

Balkhi, M. H. and Yousuf, A.R. 1992. Community structure of crustacean plankton in relation to trophic conditions. *International Journal of Ecology and Environmental Science*,18:155-168.

Balkhi, M. H. and Yousuf, A.R. 1996. Distributional pattern of cladoceran plankton in the fresh waters of Kashmir. *Oriental Science*, 75-81.

Banday, M., Dhar, J.K., Aslam, S., Sabia, Q. and Irfan, A. 2012. Contamination of fresh water fish "*Schizothorax niger*" with chlorpyrifos from "Dal Lake" basins, India. International *Journal of Pharma Sciences and Research*, 12:535-542.

Banday, M., Dhar, J.K., Aslam, S., Sabia, Q., Tariq, J. and Bhavna, G. 2012. Pesticide Residues In Blood Serum Samples from Inhabitants of "Dal Lake" hamlets „ in Jammu & Kashmir, India (2008-2010). *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 1: 26-31.

Bennett, R.O. and Dooley, J.K. 1982. Copper uptake by two sympatric species of killi fish Fundulus heteroclitus (L.) and L. majalis (Walkbaum). *Journal of Fish Biology*, 21: 381–398.

Beyers, D.W., Rice, J.A., Clements, W.H. and Henry, C.J. 1999. Estimating physiological cost of chemical exposure: integrating energetics and stress to quantify toxic effects in fish. *Canadian Journal of Fisheries and Aquatic Science*, 56: 814–822.

Beyersmann, D. and Hartwig, A. 2008. Carcionogenic metal compounds: recent insight into molecular and cellular mechanisms. *Archives of Toxicology*, 82:493–512.

Bogoni, J.A., Armiliato, N., Araldi-Favassa, C. and Techio, V.H. 2014. Genotoxicity in *Astyanax bimaculatus* (Twospot Astyanax) exposed to the waters of Engano River (Brazil) as determined by Micronucleus Tests in erythrocytes. *Archives of Environmental Contamination and Toxicology*, 66:441–449.

Bolognesi, C. and Fenech, M. 2012. Mussel micronucleus cytome assay. *Natural Protocols*, 7: 1125–1137.

Cavas, T. 2011. In vivo genotoxicity evaluation of atrazine and atrazine–based herbicide on fish *Carassius auratus* using the micronucleus test and the comet assay. *Food and Chemical Toxicology*, 49:1431-1435.

Cavas, T. and Konen, S. 2008. *In vivo* genotoxicity testing of the amnesic shellfish poison (domoic acid) in piscine erythrocytes using the micronucleus test and the comet assay. *Aquatic Toxicology*, 90:154–159.

CSIR-NEERI Report. 2013. *Interim* Report -II: Water Quality Assessment of Dal-Nigeen Lake.

Dar, S.A., Yousuf, A. R., Balkhi, M.H., Ganai, F.A. and 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.

Dar, S.A., Yousuf, A.R., Balkhi, M.H., Ganai, F.A. and Bhat, F.A. 2014. Investigation of the genotoxicity of endosulfan to freshwater cyprinid fish crucian carp (*Carassius carassius* L.) using the micronucleus and chromosomal aberration as biomarkers. *Nucleus*, 57:87-98.

Edmondson, W. T. 1961. Changes in Lake Washington following an increase in the nutrient income. *Verhandlungen Internationale Vereinigung Limnologie*, 14:167-175.

Ergene, S., Cavas, T., Celik, A., Koleli, N. and Aymak, C. 2007. Evaluation of river water genotoxicity using the piscine micronucleus test. *Environmental and Molecular Mutagenesis*, 48:1- 9.

Fenech, M. 2000. The in vitro micronucleus technique. *Mutation Research*, 455:81–95.

Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland., N., Bonassi, S. and Zeiger, E. 2003. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 534: 65-75.

Hose, J. E. 1994 . Large scale genotoxicity assessment in the marine environment. *Environment Health Perspectives*, 102: 29–32.

Hose, J. E., Cross, J.N., Smith, S.G. and Diehl, D. 1987. Elevated circulating erythrocyte micronuclei in fishes from contaminated sites off Southern California. *Marine Environmental Research*, 22: 167-176.

Izquierdo, J. I., Machado, G., Ayllon, F., d'Amico, V.L. and Bala, L.O. 2003. Assessing pollution in coastal ecosystems: A preliminary survey using the micronucleus test in the mussel *Mytilus edulis*. *Ecotoxicology and Environmental Safety*, 55: 24–29.

Lemos, C.T. and Erdtmann, B. 2000. Cytogenetic evaluation of aquatic genotoxicity in human cultured lymphocytes. *Mutation Research*, 467: 1–9.

Lemos, C.T., Rodel, P.M., Terra, N.R., Olivera, N.C.D. and Erdtmann, B. 2007. River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. *Ecotoxicology and Environmental Safety*, 66: 391–401.

Linde-Arias., Inacio, A.F., Leonardo, A., Novo, L.A., Alburquerque, C.D. and Moreira, J.C. 2008.

Multibiomarker approach in fish to assess the impact of pollution in a large Brazilian river, Paraiba do Sul. *Environmental Pollution*,156: 974–979.

Minissi, S., Ciccotti, E. and 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 fresh water. *Mutation Research*, 367: 245–251.

Nwani, C D., Lakra, W.S., Nagpure, N.S., Kumar, R., Kushwaha, B. and Srivastava, S.K. 2010. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa Punctatus* (Bloch). *International Journal of Environmental Research and Public Health*, 7:3298–312.

Ohe, T., Watanabe, T. and Wakabayashi, K. 2004. Mutagens in surface waters: a review. *Mutation Research*, 567: 109–149.

Ohle, W. 1956. Bioactivity, production, and energy utilization of lakes. *Limnology and Oceanography*, 1: 139–149.

Pandit, A.K. and Yousuf, A.R. 2002. Trophic status of Kashmir Himalayan lakes as depicted by water chemistry. *Journal of Research and Development*, 2: 1–12.

Rawson, D.S. 1939. Some physical and chemical factors in the metabolism of lakes.In Problems of Lake Biology. *Publication of the American Association for the Advancement of Science*, 9–26.

Reimer, L. 1985. Scanning Electron Microscopy: Physics of Image Formation and of Microanalysis. *Springer-Verlag*, *Berlin*, FRG.

Saleh, K. and Zeytinoglu, H. 2001.Micronucleus test in peripheral erythrocytes of *Rana ridipunda* as an indicator of environmental pollution. *Anadolu University Journal of Science and Technology*, 2: 77-82.

Tagliari, K.C., Cecchini, R., Rocha, J.A.V. and Vargas, V.M.F. 2004. Mutagenicity of sediment and biomarkers of oxidative stress in fish from aquatic environments under the influence of tanneries. *Mutation Research*, 561: 101–107.

Tan, F., Wang, M., Wang, W. and Lu, Y. 2008. Comparative evaluation of the cytotoxicity sensitivity of six fish cell lines to four heavy metals in vitro. *Toxicology in Vitro*, 22:164–170.

Vargas, V.M.F., Miglia vacca, S.B., Melo, A.C., Horn, R.C., Guidobono, R.R., Ferreira, I.C.F. and Pestana, M.H.D. 2001. Genotoxicity assessment in aquatic environments under the influence of heavy metals and organic contaminants. *Mutation Research*, 490:141–158.

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

Vine, M. F. 1990. Micronuclei. In: "Biological markers in epidemiology", B.S. Hulka., T.C. Wilcosky and J.D. Griffith. Oxford University press, New York, 125–146.

Zapata, L.M., Bok, B.C., Orozco, L.Y. and Palacio, J.A. 2016. Application of the micronucleus test and comet assay in *Trachemys callirostris* erythrocytes as a model for *in situ* genotoxic monitoring. *Ecotoxicology and Environmental Safety*, 127:108–116.

Zargar, U.R., Yousuf, A.R., Mushtaq, B. and Jan, A. 2012. Length–Weight Relationship of the Crucian carp, *Carassius carassius* in Relation to Water Quality, Sex and Season in Some Lentic Water Bodies of Kashmir Himalaya's. *Turkish Journal of Fisheries and Aquatic Sciences*, 12: 683-689.

****