

STUDIES ON CHROMOSOMAL ABNORMALITIES INDUCED IN FRESHWATER FISH *CHANNA PUNCTATUS* BY POLLUTANTS PRESENT IN WATER SAMPLES OF RAMGARH LAKE

***Kumari Mamta and Ajay Singh**

*Department of Zoology, D.D.U. Gorakhpur University,
Gorakhpur- 273009 (U.P.) India*

**Author for Correspondence: singhajay_gkp@rediffmail.com*

ABSTRACT

Ramgarh lake is an important natural lake of Gorakhpur, U.P., India, which receives huge amount of effluents from different sources without treatment. This induces alteration in the water quality as well as induces the genotoxic effects on aquatic animals. In this study chromosomal aberration in liver cells of *Channa punctatus* was conducted to estimate the effect of genotoxicity of water pollutants present in this lake. Result showed the significant ($p < 0.05$) changes in chromosomal structure i.e. chromatid breaks, fragments, gap, chromatid separation, deletion and ring type chromosomes in the liver cells of fish. This study indicates that the effluents released in lake without any treatment causes genetic damage at chromosomal level.

Keywords: *Ramgarh Lake, Chromosomal aberration, Channa punctatus, Genotoxicity*

INTRODUCTION

Water bodies which receive huge amount of toxic substances, among which pollutants released from domestic waste, industrial and other man-made activities are of significant importance as the pollutants cause toxicity, bioaccumulation potential and ability to induce damage in DNA. The study of genotoxic effect of effluent discharged and other pollutants in fishes is very important, some workers (Rishi and Grewal, 1995; Arockia-Rita and Selvanayagam, 1998; Chauhan *et al.*, 2000; Devi *et al.*, 2001; Aboul, 2002; Poli *et al.*, 2003; Lioi *et al.*, 2004; Saxena and Rana, 2005 and Ali *et al.*, 2008) have reported genotoxic effects of some pesticide and other pollutants on mammals and fishes. Genetic toxicology is a multidisciplinary way of research work involved in detecting components which is responsible for causing DNA damage and protecting DNA, With the aim of understanding potential biological consequences and molecular mechanisms of genetic material (Uhl *et al.*, 2003). The changes in genetic materials in aquatic organism caused by genotoxins, can be detected in fish at specific level by using various genotoxic assay. Fishes used in the form of suitable model for monitoring aquatic genotoxicity because of their ability to metabolize xenobiotics and accumulate pollutants (Grisolia and Cordeiro, 2002). Fishes can also respond to mutagens at low concentration of toxicants in a manner similar to higher vertebrates (Al-Sabti and Metcalfe, 1995). Chromosomal aberration analysis has been demonstrated as sensitive indicator of DNA damage caused by environmental pollutants by several workers using aquatic models (Klingerman *et al.*, 1975; Alink *et al.*, 1980; Hooftman, 1981; Harrison *et al.*, 1986; Yadav and Trivedi, 2006). The study of chromosomal aberration test (CAT) in Chromosomes of kidney cells in fishes is most suitable to observation the genotoxic effect of pesticides. Synthetic pyrethroid are more highly toxic for fishes because it is absorbed by the gills in low concentration of water due to their high lipophilicity (Saxena and Seth, 2002; Seth and Saxena, 2003, Sirohi and Saxena, 2006 and Saxena and Sirohi, 2007). These compounds possess moderate mammalian toxicity but they are extremely toxic to fish and aquatic invertebrates (Eisler, 1992). Ramgarh Lake is an important water body of Gorakhpur, U.P. India, which received untreated huge amount of effluents from different site of Gorakhpur city. It covers an area of 723 hectares (1,790) with a circumference of 18 Km long. On the southeast of Gorakhpur city and south side of the metalled road to the Kasia in Deoria district is the Ramgarh Lake. In this study the experiment were

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conducted to estimate the effect of effluents induced genotoxicity in freshwater fish *Channa punctatus*. The aim of this study was to evaluate the effects of pollutants on genotoxicity in freshwater fish *Channa punctatus*.

MATERIALS AND METHODS

Description of study area and effluents discharged

The present study area is Gorakhpur situated in the east of Uttar Pradesh, India on National highway-28 lies between Latitude 26° 46' N and Longitude 83° 22' E, cover the geographical area of 3483.8 sq. Ramgarh Lake is a large natural lake situated southeast of Gorakhpur in eastern Uttar Pradesh. It lies within the floodplain of Rapti River and outflow into it through a drain called Gurrah Nalla. The lake had maximum water spread of about 723 hectares in 1970s but has now shrunk to 678 hectares. Its maximum water depth has also declined from 4.5 m in 1990s to less than 3.5m at present. Now day Ramgarh Lake has more polluted because it receives huge amount of effluent discharge from different sites and its effects on flora and fauna of lake. These pretreated effluents from the remainder of the different sources are collected into a single drain and release into the Ramgarh Lake.

Selection of sites

Selection of site on the basis of effluent discharged in Ramgarh lake. These sites were used for caught fishes and measurement the effects of effluents on genotoxic level. There are three different sites given below:-

Site 1: Padley Ganj Nalla Gorakhpur, U.P.

Site 2: Bichhiya Nalla railway colony Gorakhpur, U.P.

Site 3: R.K.B.K. Maruti servicing centre Kunraghat Gorakhpur, U.P.



Fig 1. Padley Ganj Nalla Gorakhpur, U.P.



Fig 2. Bichhiya Nalla railway colony Gorakhpur, U.P.

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Fig 3. R.K.B.K. Maruti servicing centre Kunraghat Gorakhpur, U.P.

Collection of fish-Took ten healthy fish *Channa punctatus* (Bloch, Family- Channadae and Order- Channiformes) of an average total length of 15 ± 0.56 cm and average weight of 100 ± 2.84 gm were brought to laboratory from Government hatchery Chhapia, Gorakhpur (U.P.). These acclimatized fish were used for experiments.

Experimental design- Potential effect of effluents was studied in the form of chromosomal aberration parameters in the sets of two experiments:-

- 1-Fishes caged in pollution free water i.e. control group
- 2-Fishes caged in polluted water collected from different sites of Ramgarh lake i.e. experimental fish.

Chromosomal aberration test

After one week of treatment chromosomal aberration test (CAT) was done by method of Nagpure *et al.*, 1997. Took a healthy fish (20 cm length and 60-180 g weight) and injected with prepared Colchicines injection on caudal vein and kept for 1.5-2 hrs after injecting. Then anesthetize fish and dissect out liver and muscles tissue, homogenized in hypotonic solution in homogenizer. Pour the cell suspension in 15 ml centrifuge tube and incubate for 20-25 minutes at room temperature. After incubating solution utilized conroy's fixative for stop hypotonic action for 30 min or overnight. Removed supernatant with pipette and slowly over layer 6-8ml add chilled fixative. Then supernatant is discarded and tissue were dropped on clean slide and make thin film by the help of glass slides. For permanent slide it stain with 4-5% of Geimsa in phosphate buffer (pH 6.8), after staining slide washed with DDW and air-dried. Make permanent preparation by mounting with DPX, screen in oil immersion objection (100x).

RESULT AND DISCUSSION

The data of physical and chemical parameters of polluted water body were discussed (Table-1) on the basis of three seasons i.e. summer, rainy and winter season. According to physico-chemical parameters result was shows significant alteration when compared to General standard by central pollution control board of India (CPCB). The result of chromosomal aberration and Mitotic index (MI) analysis of effluents for different season is given in (Table-2). Present data has been focused on the chromosomal aberration (CA) caused by different effluents; which induced the formation of Chromosomal break, Chromatid braek, fragment, Chromosomal ring and deletions. According to different types of aberration, site-1is more polluted. Site-1 have highest Mitotic index (MI) in summer, rainy and winter season that is 19.5 ± 1.62 , 11.5 ± 0.69 and 8.88 ± 0.65 , respectively and it is also highest mitotic index as comparison to control group. In other hand in Site-2 and site-3 observed mitotic index 19.5 ± 1.03 , 9.77 ± 0.60 , 8.44 ± 0.64 and 15.5 ± 1.66 , 7.10 ± 0.99 , 7.55 ± 0.28 in summer, rainy and winter season respectively. In each site observed highest value of MI in summer season and lowest in winter season. In present investigation changes in liver cells of *Channa punctatus* have been observed due to huge amount of pollutants concentration received from polluted areas. The changes in chromosomal structure directly affects the genetic material (DNA) of the animals and by examining the effects of discharged on structure of

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chromosomes, some pesticide have clastogenic properties which also affect on genetic material (Ali *et al.*, 2009). In epidemiological studies, the elevated frequencies of chromosomal aberrations (CA) of peripheral blood lymphocytes have significantly elevated risk of developing cancer (Bonassi *et al.*, 2000; Obe *et al.* 2002). Chromosomal aberration is the small fraction of DNA due to changes in genetic material by the action of xenobiotics materials and affects an enormous plasticity of genome, has far-reaching consequences for evolutions (Caporale, 1999). In initial stages in fishes formation of aberration in genetic materials due to action of ethylenethiourea (ETU) but later on with increase in effluents in water fish developed a mechanisms by which they accumulated ETU in their body and CA formation fail to divided and multiply. According to (Bolognesi *et al.* 2006) well documented that the age is significantly role play in increasing of MN (micronuclei) and CA (chromosomal aberration). During S-phase, the DNA molecules are extremely longer and fibrillar structure. Other hand in metaphase chromosome its length about 10µg. These chromosomes are associated with different types of protein packaging. Due to their enormous dimensions, DNA molecules in chromosome are permanent targets of chemical and physical of diverse origin such as chromosomal aberration (Obe *et al.* 2002). According to (Bryant *et al.* 1998) experimental observation has been shows that CA, in fishes and other organism, induced by strand breaks (DSBs). DSBs are not directly able to induced chemical mutagens but lead to other lesion in chromosomal DNA, which during repair or DNA synthesis may give and rise to DSBs and eventually to CA. Thus result of the present study was revealed that effluents can cause serious effect on genetic material, when present in the water even at sub lethal concentration. The result also indicate that the frequency of chromosomal aberration in fish serve as a tool to assess the existence of genotoxic pollutants. Large amounts of effluents discharged in water body without any treatment, it is change the quality of physico-chemical parameters as well as causes genotoxicity in fishes (Mamta and Singh., 2017).

Table 1: Physico-Chemical characters of water samples collected from different sites of Ramgarh lake in different season (Mamta and Singh, 2017)

Characteristic	Season	Site-1	Site-2	Site-3	General standard by central pollution control board of India
Temperature	Summer	30.0±0.03	29.9±0.09	29.9±0.012	Shall not exceed 5°c above the receiving water temperature
	Rainy	25.0±0.06	26.8±0.15	26.2±0.02	
	Winter	17.5±0.03	15.6±0.07	17.6±0.10	
Ph	Summer	8.5±0.06	6.9±0.03	7.0±0.09	5.5-8.5mg/L
	Rainy	8.0±0.06	7.0±0.03	8.0±0.06	
	Winter	7.6±0.10	7.4±0.07	7.0±0.13	
DO mg/L	Summer	7.3±0.02	9.8±0.02	10.5±0.02	30mg/L
	Rainy	8.5±0.01	10.2±0.01	10.3±0.06	
	Winter	8.3±0.03	10.3±0.03	10.4±0.03	
BOD mg/L	Summer	473±1.722	277±2.314	76.8±0.983	30mg/L
	Rainy	482±1.453	288±2.887	77.5±0.345	
	Winter	501±1.145	235±1.577	72.1±1.201	
COD mg/L	Summer	1126.6±1.146	430.8±1.640	425±2.145	250mg/L
	Rainy	1180±2.886	681±7.264	291±4.509	
	Winter	1210±2.856	456±4.409	254±1.527	

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Table 2: Showing frequency of chromosomal aberration induced by pollutants in liver cells of *Channa punctatus* in summer (May to June 2015), rainy (August to September 2015) and winter (November to December 2015) season.

Group	Season	Total no. of Metaphase analysed (n ₁)	Metaphase with CA	Types of CA						% Aberration (n ₂)	Mitotic Index (n ₂ /n ₁)
				A	B	C	D	E	F		
Control	Summer	150	7	1	1	2	0	1	2	4.66	3.10±0.28
	Rainy	150	8	0	2	3	1	1	1	5.33	3.55±0.38
	Winter	150	5	2	0	1	0	0	2	3.33	2.22±0.36
Site-1	Summer	150	44	4	10	6	15	5	4	29.33	19.5±1.62
	Rainy	150	26	2	4	6	7	4	3	17.33	11.5±0.69
	Winter	150	20	2	2	6	5	3	2	13.33	8.88±0.65
Site-2	Summer	150	42	3	7	9	11	8	4	28.33	19.5±1.03
	Rainy	150	22	2	6	5	4	2	3	14.66	9.77±0.60
	Winter	150	19	1	6	4	3	2	3	12.66	8.44±0.64
Site-3	Summer	150	35	3	6	14	7	3	2	23.33	15.5±1.66
	Rainy	150	16	2	8	2	3	0	1	10.66	7.10±0.99
	Winter	150	17	3	2	4	3	2	3	11.33	7.55±0.28

- *A- Chromosome gap B- Chromosome break C- Chromatid deletion D- Chromatid breaks E- Fragment F- Ring Chromosome.*
- *Significant (p<0.05) when student's test was applied between treated and control group.*

CONCLUSION

It is clear from above study that Ramgarh lake got polluted due to effluents discharged from different sources of Gorakhpur city and this pollution has adversely affected the aquatic fauna as well as communities in surrounding areas, economically depended on this water body for fishing and other purposes. However the seriousness of this problem has been realized recently but not much work has been done on the toxic effects of effluents on the water quality and fish fauna present in Lake. Hence, an attempt was made to assess the toxic impact of effluents on water quality and chromosomal aberration parameters of fish caged in pollution water from different sites of water body. So it should be believed that the data obtained from this study will provide baseline information for making effective fishery conservation programmed in this area.

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