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International Journal of Current Research
Vol. 4, Issue, 11, pp.253-256, November, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

GENOTOXIC POTENTIAL OF CHANNA PUNCTATUS ON RIVER COOUM, CHENNAI

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ARTICLE INFO

Article History:
Received 22th August, 2012
Received in revised form
25thSeptember, 2012
Accepted 16th October, 2012
Published online 30th November, 2012

Key words:
Chromosomal,
Tissues, Cooum water,
Channa punctatus.

ABSTRACT

A study was conducted to assess the cytogenetic changes in an air breathing fish, *Channa punctatus* subjected to the polluted water of River Cooum. A control group of fish was maintained without any treatment. The fishes were exposed to Cooum water as test samples for 96 hours. The opercular movements were counted at an interval of 1 hour, four times a day for both the control and experimental. The tissues of gill and kidney were then subjected for the study of chromosomal aberration. It was found that the fishes which were exposed to Cooum water showed a decreasing rate of opercular movements as compared to the fishes exposed to tap water. Several structural chromosomal aberrations such as chromatid breaks and gaps, fragments, endoreduplication, ploidy were observed in the fishes subjected to Cooum water in comparison to the Control fishes thus indicating induced mutation in fishes subjected to Cooum water.

INTRODUCTION

Water pollution is a global problem, affecting both the industrialized and the developing nations. Water pollution is caused by various human activities such as mining, agriculture, stock breeding, fisheries, forestry, and urban human activities, manufacturing industries, construction works and various tertiary industries (Sinha, 1998). The pollution situation in India is worse than some of the highly industrialized countries of Western Europe and America. Most of the Indian rivers, stream and ponds are severely polluted or act as 'open sewers', sewage and industrial wastes either treated or partially treated are allowed to be discharged into the rivers and seas (Selvaraj *et al*., 2001). The Cooum River in the city of Chennai gets polluted due to letting in of sewage water by encroachers, slum dwellers and by Madras Metropolitan Water Supply and Sewerage Board at a number of locations.

About 35 per cent of the total pollution in the city lives in the slums, in which there are 38 clusters of slum settlements along the river Cooum with the population of 6,500. The river Cooum which originates near Kaveripakkam in North Arcot district, finds its way through more than a dozen towns before entering Chennai. The length of the river is about 75 km including the Cooum group of tanks. Generally speaking only the course lying within the city limits appear to be polluted. Fishes have been reported to be useful genetic models for the evaluation of pollution in the aquatic ecosystems (Mitchell & Kennedy, 1992; Park *et al.,* 1993). Freshwater fish show greater susceptibility to chemical pollution with corresponding variation in the chromosomal structure and number. Chromosome manipulation in fish is an interesting aspect, as it can be used to assess the sub lethal effect of the pollutants on living organisms (Bhagawati and Rath 1982). Genotoxic studies in different species of fish using cytogenetic analysis have been reported by a number of workers (Das and Nanda, 1986, Al-Sabti *et al.,* 1994; Al-Sabti and Metcalfe, 1995; Rishi and Sunita, 1995; Ansy Mathew and Jahageerdar 1999; Clarice *et al*., 2001; Maples and Bain 2004). Exposure of fish to pollutants and toxicants for a prolonged period, even at low levels, leads to chromosomal aberrations including gene changes (Nalini *et al*., 2009). Historically, both population and aquaculture genetics were based on superficial phenotypes or material characteristics having complex polygenetic inheritance and considerable environmental influence variations. Majority of the earlier studies recorded contain grossly inaccurate reports of possessing diploid chromosomes in species of fish. However most fish species have either large number of very small chromosomes which are difficult to spread and count accurately or a very small number of large chromosomes. The cytogenetic methodologies offer best opportunities for detecting the effects of genetically active substances on the genome of fishes.

MATERIALS AND METHODS

The *Channa punctatus* a common riverine fish belong to the family Channidae, found in the state of Tamil Nadu, India and it is commonly known as (Kuravai) snake head fish has been chosen for the present study. Water samples were collected from three different sites of river Cooum in Chennai i.e Choolaimedu, near Taj Connemara, Anna Salai and from near University of Madras Chepauk. Physicochemical parameters namely pH, total suspended solids, total dissolved solids, chlorides, sulphates, BOD, COD, sulphites, ammoniacal nitrogen, phenolic compounds, copper, zinc, lead, nickel,

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cadmium and total chromium were analyzed and were cross verified with the Tamil Nadu Pollution Control Board, Guindy. Fishes (*Channa punctatus*) were brought to the laboratory and were acclimated for 10 days. The average lengths and weights of the fish recorded were 6.5 ± 4 cm and 15± 2 gms respectively. Then the fishes were exposed to Cooum water as test samples and in tap water as control for 96 hours. The opercular movements were counted at an interval of 1 hour, four times a day for both the control and experimental. The tissues of gill and kidney were then subjected for the study of chromosomal aberration with the conventional colchicine – sodium citrate hyponic – acetic ethanol – flame drying giemsa technique (Nalini *et al*., 2009).

Table 1. Physico-chemical characteristic of the composite water sample from river cooum

Sl.No	Parameters	Unit	Sample
1	pH		7.11
2	Total suspended solids	mg/L	1040
3	Total dissolved solids	mg/L	1568
4	Chlorides	mg/L	558
5	Sulphates	mg/L	148
6	BOD	mg/L	207
7	COD	mg/L	800
8	Sulphides	mg/L	3
9	Ammoniacal Nitrogen	mg/L	4
10	Phenolic Compounds	mg/L	<0.0005
11	Copper	mg/L	0.98
12	Zinc	mg/L	2.46
13	Lead	mg/L	0.29
14	Nickel	mg/L	0.23
15	Cadmium	mg/L	0.14
16	Total Chromium	mg/L	<0.01

<- indicates less than minimum detection limit

RESULTS

The physiochemical analysis of Cooum water shows that the total suspended solids, total dissolved solids, chlorides and COD were comparatively higher than the permissible limit (Table:1). It was found that the fishes which were exposed to Cooum water showed a decreasing rate of opercular movements as compared to the fishes exposed to tap water (Fig: 1, 2 and 3).

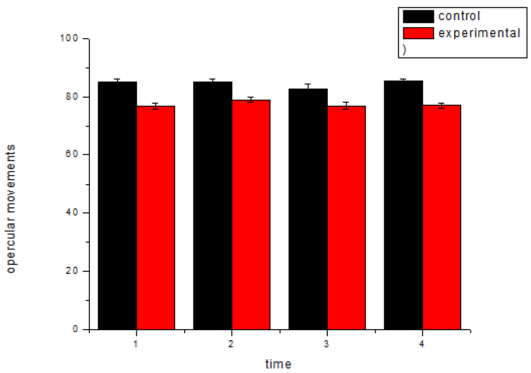

Figure 1. Opercular movements of *Channa punctatus* exposed to Cooum river water for 24h

Chromosome Analysis

The cells are subjected to microscopy for the observation of cell cycle. It is interesting to note that the karyotype of this species is easy to enumerate because it has only 16 pairs

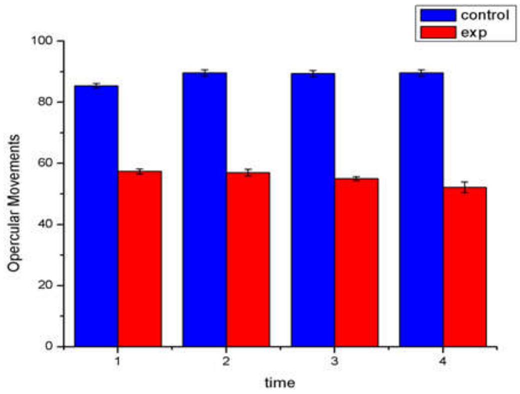

Figure 2. Opercular movements of *Channa punctatus* exposed to Cooum river water for 48h

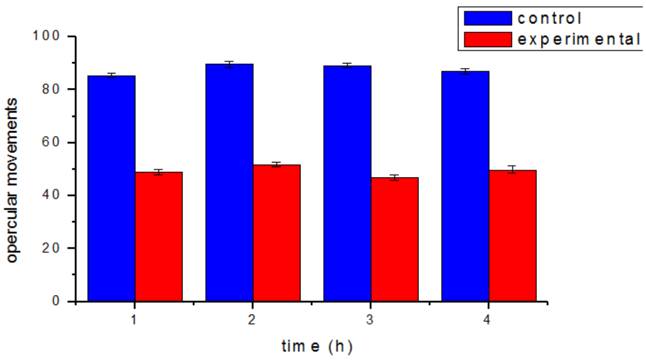

Figure 3. Opercular movements of *Channa punctatus* exposed to Cooum river water for 72h

(Fig.4) out of this 8 pairs are metacentric, 6 pairs are sub-metacentric and the remaining is acrocentric (Fig.5), for the purpose of karyotyping analysis, the fish subjected to Cooum water and tap water are administered with 0.2 colchicine and the cells of gill and kidney are isolated using stereo dissection procedure. Generally, the cells spend more time in undergoing interphase with corresponding variation in the chromosomal morphology. However, when the cells complete interphase they are destined to enter into M-phase where the cells assume rounded morphology. When the cell population is viewed under light microscope, the cells revealed chromosome with condensed outlook.

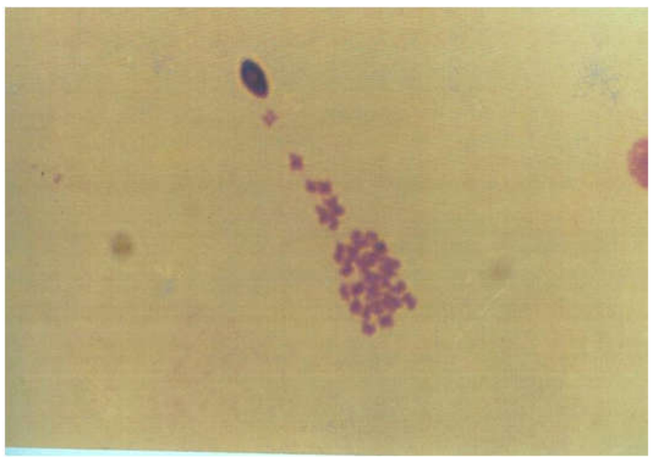

Figure 4. Chromosome of Control kidney

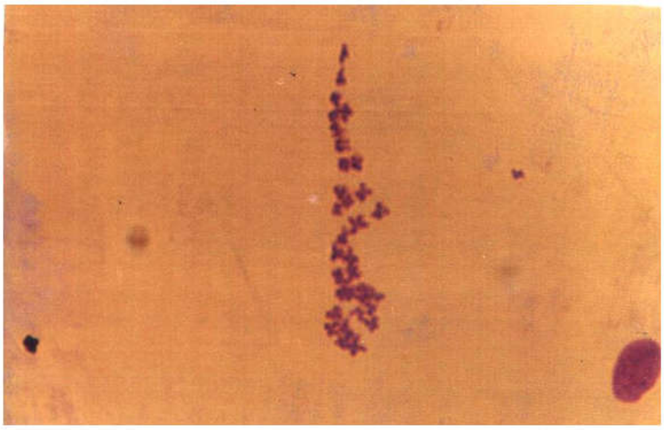

Figure 5. Chromosome of Control gill

The cells with condensed chromosome, out of the total number of observation are the direct measure of the mitotic index. In the case of gill of control fish, the incidence of mitotic spread and index are more. Relatively the mitotic index of Cooum water treated fish reveals a lesser proportions over control. Similarly the kidney cells of the control fish showed greater mitotic index with two fold increase. The control observation on the karyology of *Channa punctatus* showed a diploid value as 2n=32, consisting of 14 metacentric, 8 submetacentric, 6 subtelocentric and 4 acrocentric (Fig 4 & 5).

Chromosomal Aberration

Endoreduplication is an area of interest as there is an internal doubling of chromosome resulting from two successive DNA synthesis, a period without intervening cytokinesis and the sister chromosome are paired to form diplochromosome. However, sex linked chromosome has been given importance. Besides no difference have been detected with regard to the gross chromosomal morphology.

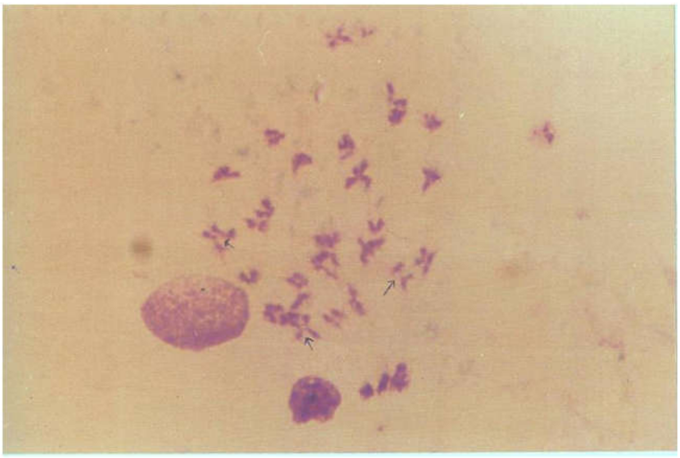

Figure 6. Chromosome of treated kidney showing Endoreduplication

However due to ploidy (Endoreplication Fig.6) and condensation, the chromosome of experimental fish appears to assume a rounded morphology. It is also interesting to note that some of the experimental kidney cells reveal less degree of ploidy with occasional breaks (Fig.7).

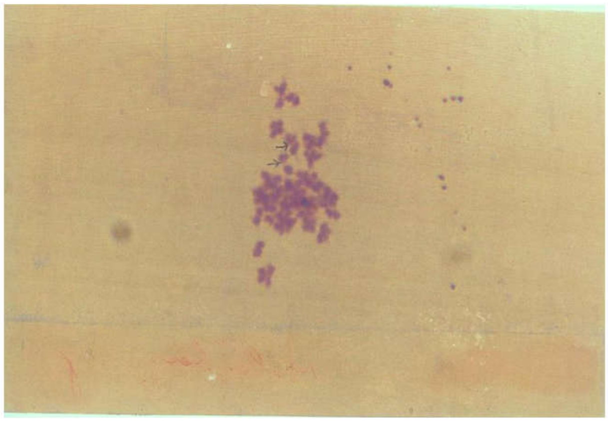

Figure 7. Chromosome of treated kidney showing Breaks (arrow indication)

Nullisomy

Changes in chromosomal number were observed to occur either by loss or addition of one set. Necessarily in *Channa punctatus* a set of chromosome are deleted showing an aneuploidic nullisomic and haploidy condition $2n^{+2}$(Fig.8 & 9).

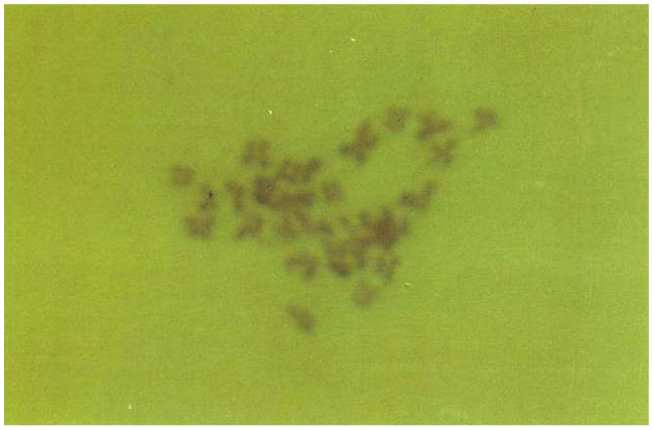

Figure 8. Chromosome of treated Gill showing Nullisomy (2N=2)

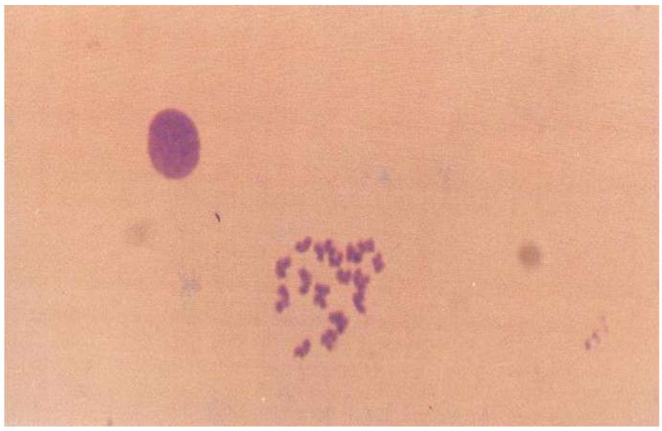

Figure 9. Chromosome of treated Gill showing Haploidy (N=16)

DISCUSSION

The analysis report of river Cooum shows a slight change in pH which indicates a minimal alteration in the hydrogen ion concentration by the entry of effluents in river Cooum. The low content of chloride in freshwater necessitates the respective salt content and it influence a lower salinity in the freshwater ecosystem. The increase in the content of metals such as copper, zinc, lead, nickel, cadmium etc in Cooum is attributed to the effluents and sewage discharge from various sources. From the nature of its physico-chemical parameters, it is clear that the Cooum water cannot retain much oxygen and hence cannot sustain organisms. Among the different parameters, nitrogen is considered as important factor which affect the survival of any organism. The increased nitrogen content of Cooum water may be due to the accumulation of more organic waste which hardens the medium and thereby it is unsuitable for sustaining aquatic organisms. The opercular movements of the experimental fish exposed to Cooum water for 24h, 48h and 72h were found to be at the average of 77.4, 55.4 and 49.3 beats per minute respectively and at each one hour interval time also there is a gradual decrease whereas the control was found to be 84.51, 87.25, and 87.8 respectively. There is a marked decrease for 24h, 48h and 72 hr exposure. The decrease in the opercular movements of the fish exposed to Cooum water compared to the experimental shows that the effluents and sewage discharged has a great effect on their metabolic rate.

The effect of various parameters of Cooum water reveals an impact on the chromosomal complement resulting in the chromosomal aberration. Colchicine interferes with the microtubules organizing centre of the metaphase cell. Even though the same concentration of colchicine is administered to the control as well as test, significant variation is observed in the chromosome of the experimental fish. Probably, the chemical constituents of Cooum must have formed a barrier around the microtubule organizing center of the gill cells; thereby the condensation of chromosome is inhibited to facilitate the mitotic index. The same technique is adopted for the kidney cells of control fish and it revealed greater mitotic index compared to the kidney cells of the experimental fish, indicating the interference of the physic-chemical factors of Cooum in the condensation of chromosome during metaphase. In the control sample the kidney cells shows increased mitotic index compared to the gill cells. This may be due to the fact that the gills are constantly exposed to the environment, while the kidney is not exposed and colchicine influenced the kidney cells more than the gill cells. The mitotic index of the kidney cells of Cooum fish showed a marked decrease compared to gill cells.

This may be attributed to the influenced of Cooum containing pollutants on the kidney cells. Polyploidy occurs during cell division because of the abnormalities. The present observation reveals a ploidy in the form of n=16 instead of 2n=32. The polyploidy in fish leads many investigators to study the causes behind. The chemistry of Cooum influenced a marked variation and which in turn is having a direct bearing on the chromosomal variation of the fish in the present study. More importantly fish undergoing ploidy has shown a faster growth rate and larger body size (Wolter *et al*., 1982). However, ploidy in fish could lead to sterility because the odd number of chromosome will interfere with the disruption of meiosis or failure of gonad development or production of aneuploid gametes. The development of gonads in triploidy is reduced or completely inhibited allowing for increase somatic growth (Wolter *et al*., 1982). The present studies also support the above view as the experimental fish showed enormous growth potential than the control fish. In nature, ploidy is possible due to the interbreeding between diploidy, tetraploidy etc. The high incidence of ploidy (endoreduplication) may be due to the diverse nature of the chromosome composition. The low availability of mitotic clones may be due to abnormality and close topographical interactions of identical chromosomal pairs (Markret, 1982). The topographical interactions may be necessary for the expression of genes and the closed interaction of these chromosomes pairs may retard or inhibit normal expression. According to the result of the present study, the chemical factors play an important role in inducing ploidy (endoreduplication), more significantly than the physical factors.

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