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Study on Genotoxic effect of agricultural and industrial effluents on chromosomes of *Channa punctatus* of polluted water bodies in West Bengal, India

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Abstract
Various fish species serve as important source of protein in the diet, so it is important to assess whether fish can serve as an early warning for potential threat both for aquatic organism as well as consumer of fish. Due to various aanthropogenic activities, different hazardous chemicals as well as heavy metals reach into water bodies through run off and ultimately affects aquatic ecosystem and it has been increased immensely over the past 50 years due to industrialization and urbanization. The present study deals with the cytological effects of effluents discharged into respective sites of rural and urban importance using *Channa punctatus* from the water as in situ sentinels by karyotyping as well as cytochrome c oxidase gene analysis. The study also aims to gain more insight into the relationship between the chromosomal differentiation and pollution. The normal number of chromosome in control group of *Channa punctatus* is 2n=32, consisting of 16 metacentric, 14 submetacentric and 2 acrocentric chromosomes. Different chromosomal aberrations like End to End joining, Chromosome Break and Gap, Pycnosis, Stubbed Arm, Acentric Fragmented chromosome, Attenuated chromosomes are found in *Channa* species collected from two polluted sites. The difference in banding pattern was found in study of cytochrome c oxidase gene PCR products which may be due to difference in nucleotide sequences resulting from point mutation or deletion or inversion or translocation.

Keywords: Water Pollution, *Channa punctatus*, Karyological study, cytochrome c oxidase gene profiling.

1. Introduction
Intensive industrial developments in last few decades have increased the concentration of a large number of chemicals in the biosphere, the habitat of all living beings, including man. Anthropogenic activities resulting from modern methods of agriculture, urbanization, industrialization involve the increased use of various types of chemical pollutants and toxicants, such as metals, biocides, pesticides, chemicals, industrial effluents, etc. which ultimately reach into aquatic environments and become responsible for the degradation of aquatic ecosystem [1,2]. The impacts of chemical compounds are also diverse depending on the quantities, toxicological and genotoxic potentials of the chemicals and at the same time the character of recipient water bodies (whether flowing or stagnant, sedimentation rate, temperature, salinity etc.). Many of these pollutants are non-biodegradable and can accumulate in the aquatic environment and in turn results biomagnifications in aquatic organisms and as well as in the consumer of aquatic products like humans. Thus they are harmful for the health of both human and other animals [3, 4].
Fish provide an excellent source of material for the study of the pollution in water samples as they are aquatic vertebrate, which can metabolize, concentrate and bioaccumulate water pollutants. In ecotoxicological studies, fish can be selected as a sensitive bioindicator of aquatic contamination particularly in tropical regions [5]. There are many cytogenetic end points that can be used as an indication of exposure to genotoxic substances in aquatic organisms. Fish chromosomes can act as an important bio-indicator of the presence of pollutant in the water. Thus chromosomes studies in fishes have become increasingly important now a day, for evaluation of genotoxic potential of pollutants. Most of the genotoxic work has been carried out on fishes using various pesticides, weedicides and herbicides [6-10]. Aquatic genotoxicity is reflected perfectly in fish as biomagnification of those pollutants and it can be easily assessed in their system. Many research works have been done to define Quantitative chemical structure versus biological activity relationship for pollutants in an

aquatic environment. Few studies have been done to ascertain the genotoxic or effects of metal ions on chromosomes [11-15].
Majority of the fishes have larger number of 2n chromosomes and the chromosomes are acrocentric type which makes it difficult to study the genotoxic effects in fishes. Several ecotoxicological characteristics of air-breathing freshwater food fish *Channa punctatus* such as its wide distribution and availability throughout the year, easy maintenance in the aquaria/wet lab, and the presence of small number (2n=32) well-differentiated diploid chromosomes make this species an excellent model for toxicity studies [27].
Cytogenetic endpoints like micronuclei formation, chromosome aberration, binucleation and sister chromatid exchange are very sensitive genetic assays for detecting genotoxic chemicals and environmental mutagens at sub-toxic levels. The binucleation is an indicator of abnormal cell division due to blocking of cytokinesis. This abnormal cell division would result in a genetic imbalance in the cells, which may also be involved in carcinogenesis [16]. A growing interest in genotoxicity caused by environmental pollutants has led to the development of several biological tests for detecting and identifying genotoxicants in the environment [17].
Different mitochondrial genes like cytochrome c oxidase gene, a conserved gene, can be used for population genetics and genotoxic study. Recently, the study of Parson [18] proposed the identification of vertebrate species by nucleotide sequence analysis of the cytochrome c oxidase gene. The cytochrome c oxidase gene is one of the 37 genes within the circular mitochondrial genome [19]. This gene is ideal for species identification as it shows limited variability within and much greater variation between species.
The present study deals with the cytological effects of effluents discharged into respective sites of rural and urban importance using *Channa punctatus* from the water as in situ sentinels by karyotyping as well as cytochrome c oxidase gene analysis. The study also aims to gain more insight into the relationship between the chromosomal differentiation and pollution as selected sites are greatly polluted as a result of discharging agricultural drains (the rural area pond) with insecticides, pesticides and the urban sewage canal with a flow of domestic and industrial wastes.

2. Materials and methods

2.1. Areas studied and collection of sample: The study was performed on three groups of fish (*Channa punctatus*) of 130±10 g body weight. Control group (Group A) fish were collected from Naihati fish Farm by bottom trap nets. Polluted groups include Group B and Group C.
Group B fish were collected from water body of Barasat, North 24 Parganas in the state of West Bengal, India (Fig 1) and it is a rural area based on villages and agricultural fields. Pesticides, herbicides, chemical fertilizers, organic manures as well as domestic sewage are sources of pollution in this water body. This pond is also used for washing and bathing of domestic animals.
Group C fish were collected from Dunlop, Kolkata in the state of West Bengal, India (Fig 1). Many industrial and power plants use this water body to dispose of waste materials. Water becomes contaminated with toxic materials from industry and abandoned hazardous waste sites as well as with domestic wastes.
Fishes from polluted (water body of Barasat and Dunlop) and unpolluted (Naihati Fish Farm) area were collected to study the following parameter.

Fig 1: a) Map of India showing West Bengal; b) Map of West Bengal showing the study area**.**

2.2. Karyotype Analysis and Ag NOR Staining
Live specimens of *Channa punctatus* were collected from the Naihati Fish Farm and kept them for few days in the aquarium. Chromosome preparation was obtained from gill and head kidney tissues using the technique described by Khuda-Bukhsh and Barat 1987 [20] with few modifications. Silver staining of the nucleolar organizer region was performed according to Ploton *et al*. 1986 [21].
Chromosome morphology was determined on the basis of arm ratios as proposed by Levan *et al*. 1964 [22]. Metacentric (M), submetacentric (SM), and subtelocentric (ST) chromosomes were considered as bi-armed.
For each fish group, 50 well spreaded metaphases were examined for kidney.

2.3. DNA isolation and Cytochrome c oxidase gene analysis
Genomic DNA was isolated from fish scales using the protocol of Wasko *et al*. 2003 [23]. After ensuring complete solubility of DNA, the purity factor (A_{260}/A_{280} nm) was measured spectrophotometrically and its integrity was checked.
Polymerase chain reaction (PCR) was conducted in a thermo-cycler (Applied Biosystems) in 50 µl of the reaction medium containing10x PCR Buffer (Genei, Bangalore); 50 mM KCl; 2 mM $MgCl_2$ (Genei, Bangalore); 0.2 mM of each dNTPs (Genei, Bangalore); 1 mM primer; 20-50 ng DNA template; and 1 U of *Taq (Thermophilus aquaticus)* polymerase. The

amplification cycles were repeated 30 times and comprised denaturation (94 °C, 1 min), annealing (55 °C, 1 min), and elongation (72 °C, 2 min). Upon synthesis of amplified DNA, the reaction was terminated by decreasing the temperature at 4 °C. The amplicons were thereafter, fractionated in 1% agarose gel in 1×TAE buffer supplemented with ethidium bromide at 70-80 volt. The DNA Ladder (2000 bp- 100 bp in size) (Gene Ruler®, Fermentas) was used as molecular weight markers. One set of Forward and Reverse primers with a length of 24 bp were used for our experiment. The primers were commercially synthesized by IDT integrated DNA technologies (Genei™, Bangalore).

The primers are: Forward: 5′-TTACATAGCAGTGAAGCCCAGCCT- 3′, Reverse: 3′-AAGTAAGCGGCCAACAACGACAAC- 5′.

2.4. Water analysis

The physiochemical analyses of water were determined using standard APHA, AWWA [24-26]. Water samples were analyzed by pH meter. To determine detergent content in water, *Aromatic amine test* was done. To know the solute content dissolved in water, *salinity test*, *chlorosity test, chlorinity test* were done on the basis of the following formulae, as described by APHA, 1998 [24].

Chlorosity of water = (Volume of $AgNO_3$ consumed × Normality of $AgNO_3$) / Volume of test sample. (1)

Chlorinity of water = Chlorosity of water / Density of water (Density of water = 1) (2)

Salinity of water = [0.03+ (1.805 × Chlorinity of water)] % (3)

3. Results and Discussion

3.1. Water analysis

Table 1: Physiochemical properties of Water

Water sample	pH	B.O.D	C.O.D	Salinity	Dissolved solid	Suspened solids	Aromatic nitrogen	Chlorides	Sodium
Barasat water body	8.2	61 mg/l	266 mg/l	0.07/ppm	1342mg/l	169 mg/l	4.2 mg/l	162.0 mg/l	78.74%
Dunlop water body	5.4	56 mg/l	204 mg/l	0.08/ppm	1462mg/l	182 mg/l	3.8 mg/l	154 mg/l	57.46%

3.2. Chromosomal Aberrations

Various types of chromosomal aberrations viz. centromeric gap, chromatid break, attenuation, acentric fragment, pycnosis, stubbed arm and ring chromosomes were observed in specimens of *Channa punctatus*. The total number of aberrations and the total number of metaphases spread with chromosomal aberrations were observed to be significantly higher in the specimens of Dunlop water body than Barasat pond **(**Table 2).

Our data recorded that the structural chromosomal aberrations were more observed due to pollution than numerical chromosomal aberrations **(**Fig 2). The remarkable chromosomal aberrations recorded in the present investigation included chromatid breaks, chromosome breaks, chromatid fragments and ring chromosomes. Present study shows 6 pairs Ag-NOR region and many breaking part of chromosome, which may be due to the effect of pollution.

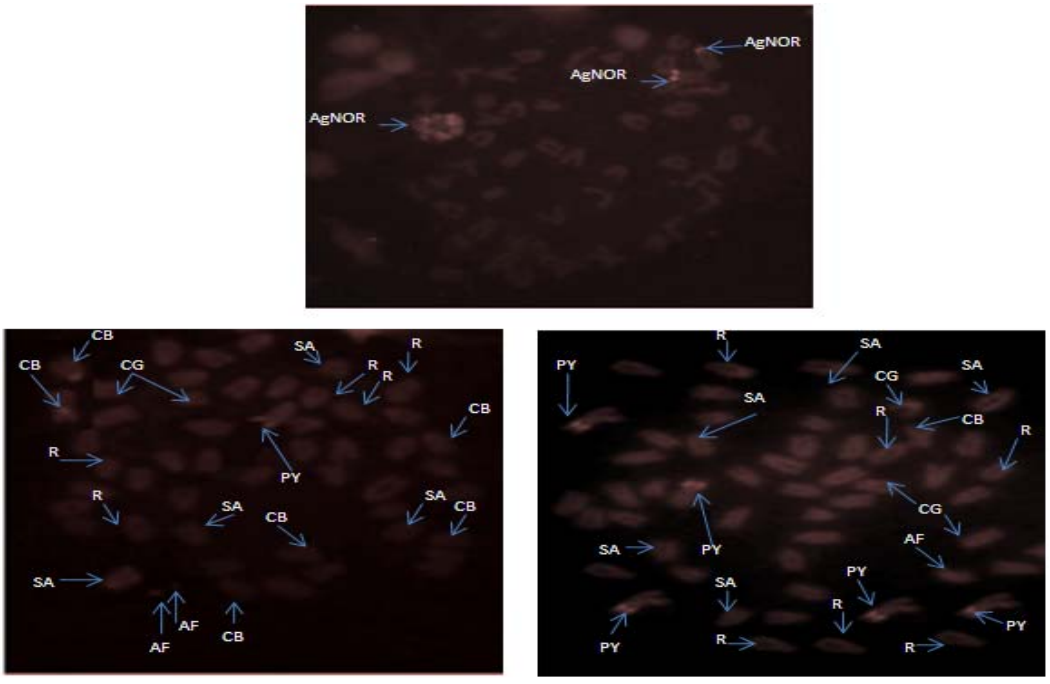

Fig 2: Somatic metaphase plate of *Channa punctatus showing* Acentric fragment (AF), Pycnosis (Py). Chromatid break (CB), Centromeric gap (CG), Stubbed arms (SA) and Ring chromosomes.

a) Control, b) Barasat water body (with domestic and agricultural sewage), c) Dunlop water body (with domestic and industrial sewage), West Bengal, India.

Table 2: Different types of Chromosomal Aberrations

Study area	Acentric frgament	Pycnosis	Chromatid break	Centromeric gap	Stubbed arms	Ring chromosome	Total no. of aberrations
Barasat	2	0	6	2	4	5	19
Dunlop	1	4	1	2	3	6	18

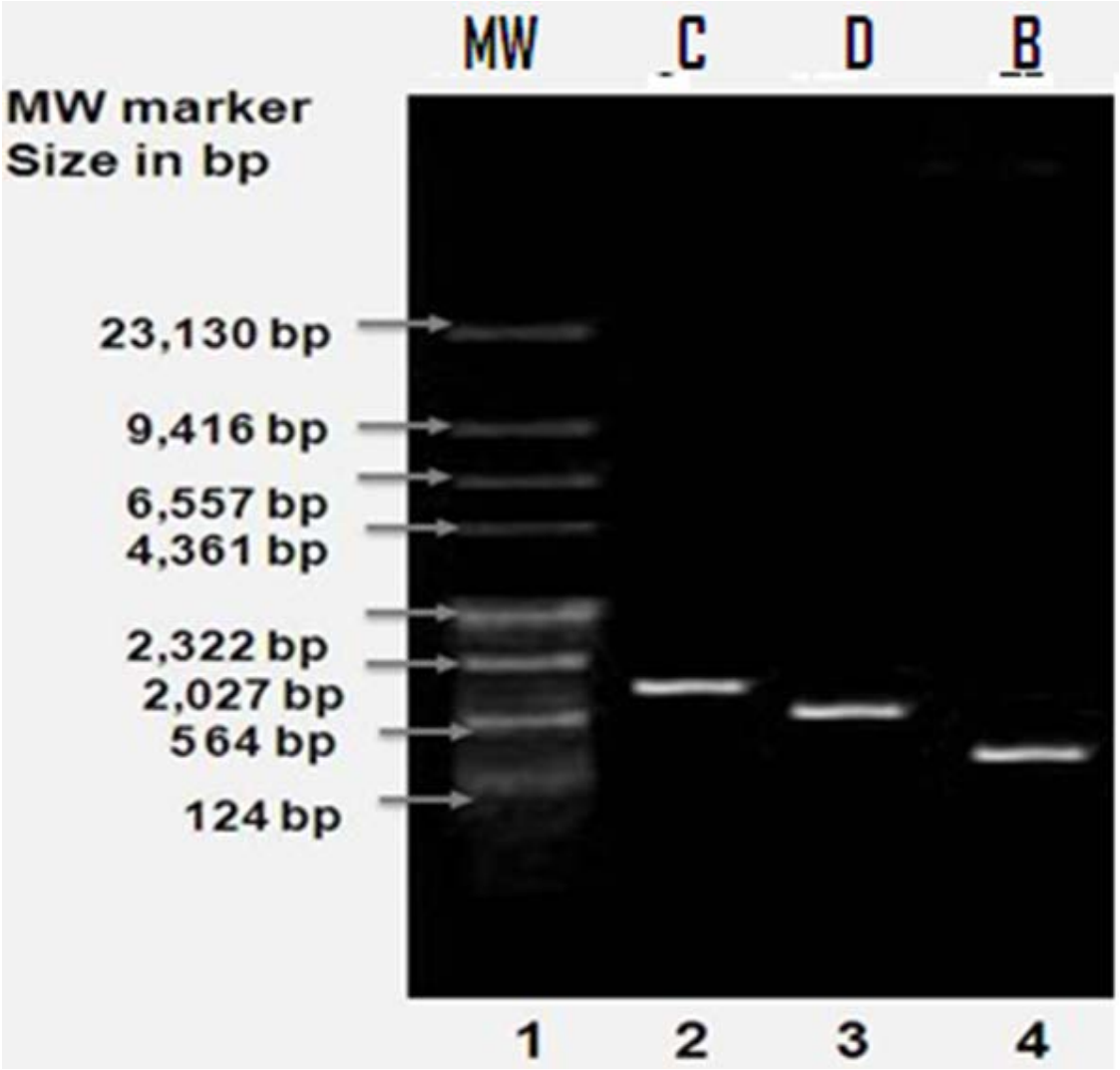

Fig 3: PCR amplification of cytochrome c oxidase gene from 50 ng of total nucleic acid of Channa punctatus using Primers. Symbols of places as in Figure and their lanes are noted above the gel micrograph. C: Control, D: Dunlop water body.
B: Barasat water body

The above gel micrograph (Fig 3) shows the cytochrome c oxidase gene profile of fish from the control and the affected areas. The bands in each lane of affected areas indicate the change in gene structure and size which may be due to deletion of some part of the gene. Further gene sequencing may give light on this area.

4. Conclusions

Fishes are very sensitive to a change in their environment and can play significant role in assessing potential risk associated with contamination in aquatic environment [27]. Mahrous and Abdou (2001) [28] studied that the environmental water pollution (agricultural waste water or industrial) have significant effects on *Oreochromis niloticus* and *Clarias lazera*, causing chromosomal aberration breaks, deletion, and centromeric attenuation in somatic cells. Mohamed *et al*. (2008) [29] detected the cytogenetic changes by measuring the frequency of chromosomal aberration in the gill cells of the treated *Oreochromis* niloticus by copper sulfate and lead acetate. Iji and Odeogan 2014 [30] studied the cytotoxic effect of effluents at inducing chromosomal aberrations, using this as a biomarker tool in wild *Clarias pachynema* for assessing and monitoring pollution of the aquatic environment.

The present results on chromosome aberrations are in accordance with the observations made by earlier workers in many fishes like *Anabas* [31], *Channa punctatus* [9, 33]; *Heteropneustes fossilis* [10]. So it has been opined that most of the chemicals produce similar aberrations which cannot be explained by a specific bio- chemical interaction between the genotoxic agents [33, 39]. Our results are also in agreement with Yadav and Trivedi (2009) [34] who found that the exposure of *Channa punctatus* (2n=32) to, mercuric chloride, arsenic

trioxide and copper sulphate pent hydrate for a Week, kidney cells revealed chromatid and chromosome breaks, chromatid and chromosome gaps, along with ring and di-centric chromosomes. The findings indicate genotoxic potential of these metals even in sublethal concentrations. Bioaccumulation and biomagnifications of persistent heavy Metals in aquatic environment were studied by Malla and Ganesh 2009 [35]. Previous research studies also indicate that Polluting substances such as heavy metals could act on organisms directly and/or form free oxygen radicals, which can initiate degenerative processes and cause genotoxic effects [36-38].

The results of present study confirm the genotoxic potential of The industrial and agricultural sewage on chromosome of fresh water fish *Channa punctatus*. Again the difference in Cytochrome c oxidase gene reveals the effect of pollutants on conserved sequence of nucleotide. The presence of aberrations correlates with the fact that DNA damage occurs for accumulation of those heavy metals on fish body. Therefore, further studies are required to arrive at a definite conclusion and to find out the active principal of genotoxicity of rural and urban sewage. Based on the results obtained, it is evident that most fish found in the two water bodies could be toxic to human if consumed for a long period of time. Therefore, a long term monitoring program of metal bioaccumulation in fish from the two water bodies would be valuable in the assessment of the potential biological risks to human health and the environment. It causes threat to fish survival and also affect the aquatic ecosystem.

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