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Research Article

ASSESSMENT OF LEAD ACCUMULATION IN MUSCLE AND ABNORMAL NUCLEATION IN THE PERIPHERAL ERYTHROCYTES OF FISH (*Mystus cavisus* HAM. -BUCH.) OF HOOGHLY RIVER DOWNSTREAM

Mayukhmala Mandal*

Department of Zoology, Charuchandra College (Affiliated to University of Calcutta), Kolkata, India

**Corresponding author: mayukhmala.cc@gmail.com*

ABSTRACT

The present study was attempted to analysis the concentration of lead (Pb) in water, sediment and muscles as well as to evaluate the frequencies (%) of several nuclear abnormalities (NA) and MN (micronucleation) in the peripheral erythrocytes of fish, *Mystus cavisus* Ham.-Buch. inhabited in the Hooghly river downstream near Birlapur, West Bengal, India. The study site was selected at Birlapur Hooghly river as downstream site. The concentration of Pb was estimated in water, sediment and muscles of studied fish species by using atomic absorption spectrophotometer and also evaluated the frequencies of different MN and NA in the peripheral erythrocytes of test fish. The results clearly indicate that the value of metal Pb as <0.1mg/L in water, 29.00 ± 9.90 mg/Kg in sediment, which are within the threshold value of international standards but14.91 ± 4.14 mg/Kg in muscle was observed beyond the international standard. The present results also reveal alarming risk of genotoxicity through the induction of MN and NA in the peripheral erythrocytes of test fish. The present study was detected Pb accumulation in the muscles of fish, which may lead to alarming genotoxic risk in the peripheral erythrocytes of studied fish. It is suggested that regular monitoring is needed for genotoxin(s) contamination level in the study area and also other sites of Hooghly river by genetic biomonitoring, which is lacking. In future, the higher risk of genotoxicity may reduce the availability of this low-cost fish.

Keywords: Pb metal; Bioaccumulation; Genotoxins; Micronucleus; Nuclear abnormalities; Genotoxicity; Riverine fish; *Mystus cavisus*

1. INTRODUCTION

The Ganga river, also called Hooghly is a highly seasonal river and it was reported that 80% of drainage discharge occurred during the monsoon (July to October) of Southwest Indian monsoon [1]. From past to recent studies, the researchers have already been documented that the discharges of untreated effluents, inputs of agricultural chemicals, discharge of organic matter and chemicals from aquaculture farms, etc. in the different sites of Ganges [1-4]. These activities lead to pollute the river during the monsoon period, mainly due to the flooding of the drainage basin [1, 3]. The water pollution by heavy metals in Hooghly river has been reviewed by Paul [3] and Bonnail et al. [1] analyzed sediments containing heavy metals in the different depth of Ganges.

The term bioaccumulation means toxicants from the media like food, water and soil are distributed and finally accumulated in the vital organs of organisms [5]. Among several organisms, fish is an edible organism and having high nutritive values.

The accumulation of metals in the vital organs of fish is matter of great concern. Among several metals, lead (Pb) is well known toxin and genotoxin, causes cancer in different tissues [6]. It was reported that in the upstream sites such as Varanasi, Allahabad, Mirzapur, and Kanpur of Uttar Pradesh, India of river Ganges heavy metals accumulation in gills, liver and muscle of inhabited fish species viz. *Cirrhinus mrigala, Cirrhinus reba, Catla catla, Lebio rohita, Crossocheilus latius, Clupisoma garua, and Mystus tengara* [7]. In downstream sites, essential and non-essential metals viz. Zn, Cu, Pb and Cd were found in the muscles of edible finfish species (*Polynemus paradiseus*, *Tenualosa ilisha*, *Liza parsia*, *Liza tade* and *Stolephorus commersonii*) collected from islands of Hoogly river and other estuaries [8].

The bioaccumulation of metals in fish lead to genotoxicity such as micronucleation (MN), abnormal nucleation (NA), DNA damage, etc. were observed by several researchers [9-13]. The study of genotoxicity in fish is a suitable indicator and the blood is important

biomarker to know easily the water pollution and the risk evaluation of toxin [14]. Few research works were done on fish genotoxicity to know water pollution at gene level of river Ganges [11-14, 15]. But in the downstream of Hooghly river, the health status especially the study of genotoxic effect of fish species is lacking.

The present study was attempted to know the concentration of Pb in water, sediment and muscle of fish as well as nuclear abnormalities in the peripheral erythrocytes of fish (*Mystus cavisus* Ham.-Buch.) inhabited in the downstream of Hooghly river, West Bengal, India.

2. MATERIAL AND METHODS

2.1. Selection of study area

The study area was selected at Birlapur site of Hooghly river, West Bengal, India. It is a part of Kolkata urban agglomeration and located at 22°25'N latitude and 88°08'E longitude.

2.2. Selection of fish species

The fish species was selected commonly known as Gulsetangra and scientific name *Mystus cavasius* (Hamilton-Buchanan, 1822) is a catfish under family Bagridae of order Siluriformes. It is commonly known as Gangetic Mystus, which has been reported to be distributed in India, Bangladesh, Pakistan, Nepal, Srilanka, Thailand and Myanmar. It is an euryomnivorous and predatory fish with wide range of food preference [16]. It has high demand as edible fish due to high nutritive value with low market price.

2.3. Collection of water and sediment

Water and sediment samples were collected from the two different zones of study area. After collection it was transported to the laboratory for analysis of lead (Pb).

2.4. Collection of fish and process for analysis

The fish *Mystuscavasius* samples were collected from nearby fish market along the river basin of Hooghly. The fishes were ranging from 10.9-12.2 cm in length and weighing between 30-35gm. All the fishes were collected just died and muscles were dissected out and kept in ziplock plastic and transported to the laboratory for Pb analysis.

2.5. Estimation of Pb

The method of Pb analysis in water, sediment and fish muscle was done as per protocol in the American Public Health Association [17]. Total Pb was estimated after digestion by concentrated nitric acid and by using an atomic absorption spectrophotometer (AAS model: Agilent Technology 200 Series AA).

2.6. Collection of blood and smear preparation

The blood was collected from heart by using insulin syringe. Total ten fish samples were studied for genotoxicity. After collection of blood the smear was prepared two slides per fish. All the slides were dried at room temperature and kept in slide box for MN and NA assay.

2.7. Assessment of MN and NA

MN and NA frequency in erythrocytes was evaluated according to Fenech [18]. All the smeared slides were used after 24h drying and fixed in 100% methanol for 10 min followed by staining with 5% Giemsa solution for 10 min, air dried and then prepared for permanent use. Total 1000 erythrocytes per slide were examined and 1000 nos. of erythrocytes were scored for each specimen under a brightfield microscope with oil immersion at 1000X magnification. MN was identified as per criteria described by Fenech et al. [19]. Other nuclear anomalies (NA) such as nuclear buds as lobed nuclei (LN), MN or micronuclei (free or attached with main nucleus) as erythrocytes bearing more than a single main nucleus, blebbed nuclei (BLN), notched nuclei (NN), nuclear fragmentation (NF), bi-nucleated erythrocytes (BN), vacuolated nuclei (VN), nuclear cariolysis (NC), Dumble shaped nuclei (DSN) and Retracted nuclei (RN) were recorded separately, as per the criteria described by Da Silva Souza and Fontanetti [20].

3. RESULTS

3.1. Pb concentration in water, sediment and accumulation in muscle of fish

The results clearly indicate (Table 1) that the value of Pb metal in river water samples were observed <0.1 mg/L as very lower concentration within the surface water quality standard prescribed by Central Pollution Control Board (CPCB), 1979 and the Bureau of Indian Standards (BIS),1982.

In case of Pb values (Mean±SD) present in the river sediment samples 29.00±9.90mg/Kg and Pb accumulation in the muscles of fish (*M. cavasius*) 14.91±4.14 mg/Kg.

Table 1: Concentration of Pb in river water, sediment (n = 4; Mean ± SD), and accumulation in the muscles of fish *Mystuscavasius* (n = 5; Mean ± SD)

Study sites	River Water (mg/L)	River sediment (mg/Kg)	Muscles of fish (mg/Kg)
Hooghly river at Birlapur site	<0.1	29.00±9.90	14.91±4.14

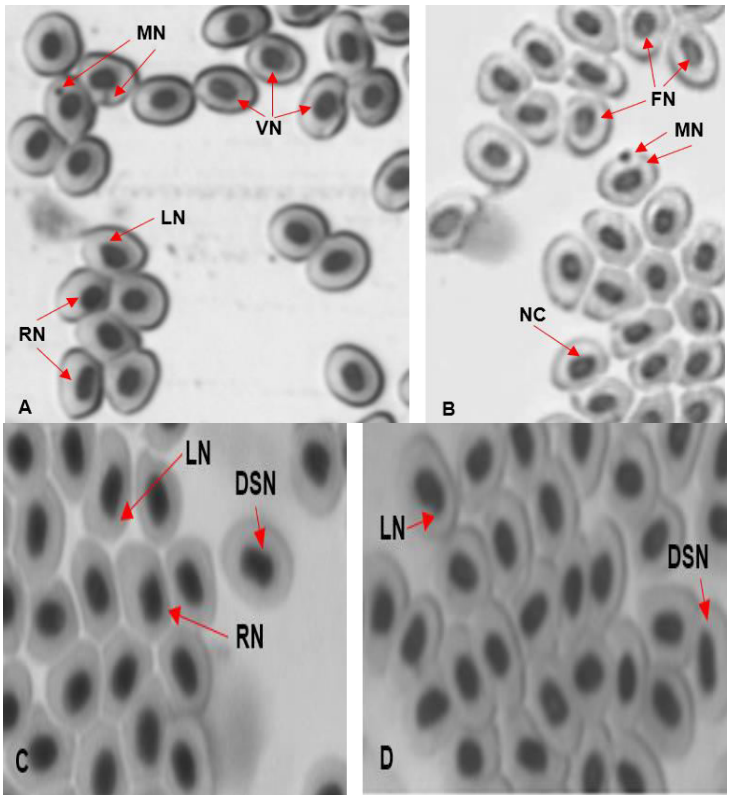
3.2. Genotoxicity especially MN and NA in peripheral erythrocytes of fish

The present results (Mean ± SD) reveal alarming risk of genotoxicity through the induction of MN and NA such as BLN, BN, NN, LN, DSN, VN, RN, NC and FN in the peripheral erythrocytes of fish *M. cavasius* Hamilton - Buchanan. The frequencies (%) of MN and different NA were observed in fish species of study site (Table 2). The microphotographs of different types of NA along with MN are depicted in Fig. 1 (A-G). In case of MN frequencies (%), the value was observed 1.64±0.32. For frequencies (%) of NA such as BLN, BN, NN, LN, DSN, RN, FN and NC values were also observed in the fishes as 1.64±0.32, 1.16±0.15, 1.02±0.25, 1.86±0.45, 3.12±0.91, 2.52±0.88, 2.61±1.22 and 3.13±1.21 respectively. The highest frequencies (%) of NA were obtained in case of NC followed by DSN, FN and RN. The frequency (%) of MN value was lower than above-mentioned NA values (Table 2).

Table 2: Percentage frequencies of MN and NA in the peripheral erythrocytes of fish *Mystuscavasius* inhabited in Hooghly river

Study sites	Genotoxic effects (in %) in fish species (n=10)								
	MN	NA							
		BLN	BN	NN	LN	DSN	RN	FN	NC
Hooghly river at Birlapur	2.01±0.68	1.64 ±0.32	1.16±0.15	1.02±0.25	1.86±0.45	3.12±0.91	2.52±0.88	2.61±1.22	3.13 ± 1.21

MN = Micronucleus; NA = Nuclear abnormalities; BLN = Blebbed nuclei; BN = Binuclei; NN = Notch nuclei; LN = Lobed nuclei; DSN = Dumble shaped nuclei; RN = Retracted nuclei;FN = Fragmented nuclei and NC = Nuclear cariolysis



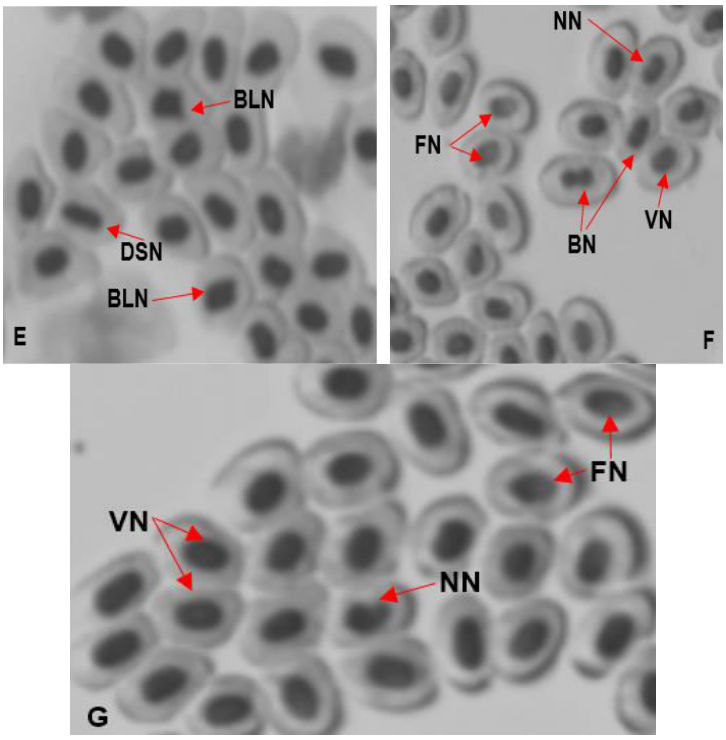


Fig 1. (A-G): Microphotographs (1000x magnification) of MN and NA in the peripheral erythrocytes of *Mystuscavasius*

(MN = Micronucleus; NA = Nuclear abnormalities; BLN = Blebbed nuclei; BN = Binuclei; NN = Notch nuclei; LN = Lobed nuclei; DSN = Dumble shaped nuclei; RN = Retracted nuclei; FN = Fragmented nuclei and NC = Nuclear cariolysis)

4. DISCUSSION

The river Hooghly is the habitat for several fish species and few species such as *Polynemus paradiseus* (Pabda), *Tenualosa ilisha* (Ilish), *Liza parsia* (Parse), *Liza tade* (Adh-Bhangone), and *Stolephorus commersonii* (Amodi) are edible have already been studied metal accumulations by Mitra and Ghosh [8] and Mitra et al. [21]. The studies of heavy metals in the Hooghly river water and sediment as well as accumulation in the vital organs of fish have been well documented by several researchers [1, 3-4, 22-24]. In recent research, it has been reported that several metals were accumulated in the vital organs of inhabited fish of river Ganga [7]. On the other hand, Sarkar et al. [25] have been reported the maximum value of dissolved Pb in water of Hooghly river recorded as 0.076 mg/ml at Station Babughat, which almost six times of the average values recorded at Station Gangasagar. An increasing trend was observed of dissolved Pb at Station Babughat and Station Diamond Harbour during monsoon period. In the present study some similarity was observed for Pb concentration in water at Birlapur as downstream of Hooghly river(Table 1) in comparison with earlier studies from other parts of river Ganges [1, 3-4] and the lower value found within the permissible limits of 0.1ppm [26] (BIS, 1991). Interestingly, the Pb accumulation in the muscle of studied fish (Table 1) found similar observations with other edible fish species inhabited in Hooghly river [8, 21], which was observed beyond the permissible limit as 2.0ppm and 0.5-6.0ppm [27-28]. In case of Pb analysis in sediment, 26.7 µg/gm of Pb was observed in Ganga river sediment at Varanasi [29] but in the present study it is an increasing trend near downstream of river Hooghly (Table 1). However, a comparison with the threshold values of 40 and 35 µg/gm by USEPA [30] and CCME [31] the sediment indicated within the permissible standards.

This fish species known as euryomnivorous, feeding on wide range of food items such as phytoplankton, zooplankton, insects and their larvae, and also different parts, roundworms and molluscans [16]. In the present study, the possibilities of Pb accumulation in the muscle is higher in the test model due to their feeding habit, which has close similarities with other fish species reported [7-8, 21]. But it was observed from earlier studies that nearly close value of Pb in case of accumulation in the vital organs of studied species may be due to chronic exposure in their life cycle.

Major studies were observed the presence of Pb in water, sediment as well as accumulation in the organs of inhabited fish in the downstream of Hooghly river but no one has been attempted to know genotoxic risk especially induction of MN and NA in the peripheral erythrocytes of this fish species when exposed to metal(s) in the river water. However, several researches have been reported in other countries of the globe as well as in India on the induction of MN and NA in the erythrocytes of fish due to metals or genotoxins [10-12, 14, 32-34].

In the present study it was observed that MN and all parameters for NA were induced in the erythrocytes of studied fish species, but the values were comparatively lower than others earlier cyto-genotoxicity studies in fish [10, 14]. Till date the mechanism of NA is not clearly understood [35] but the present study may be an indication of alarming genotoxic risk in the test fish species and this test model may be caused mutagenic to Pb or combinations with other genotoxins.

This is a first time observational study of Pb bioaccumulation followed by genotoxic risk assessment with this particular test species, but further research is particularly needed in this area with other inhabited fish species to know sensitivity of this test model and alarming genotoxic effect in the edible food like fish. Generally, individual metal or combinations of metals or chemicals may alter the nuclear shape as genotoxic stress in fish [9-12, 14, 36].

5. CONCLUSIONS

It is concluded that Pb accumulation was obtained in the muscle as well as alarming genotoxic risk especially induction of MN and NA in the peripheral erythrocytes of studied fish. The accumulation of Pb in the muscle is not good because the edible part is muscle of any fish is consumed as food. Moreover, Pb is the causative agent for bioaccumulation and showing alarming genotoxic risk but there are possibilities of cumulative effects of other metals in the water and sediment, which was not studied in the present study. It is suggested that the erythrocytes are suitable biomarkers of genotoxicity screening for this test model and regular monitoring is needed for genotoxicant(s) contamination level in the fish of river by genetic biomonitoring, which are lacking in different stations of Hooghly river. In future, study should be emphasized to know the seasonal variations of genotoxicity and the higher risk of genotoxicity, which may reduce the availability of this low-cost fish.

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Conflict of interest

No conflict of interest in the present study.

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