



# Assessment of DNA integrity through MN bioassay of erythrocytes and histopathological changes in *Wallago attu* and *Cirrhinus mirigala* in response to freshwater pollution

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## ABSTRACT

The aim of this study was to determine the level of contamination and genotoxic impact through micronucleus assay and histopathology in *Wallago attu* and *Cirrhinus mirigala* procured from the polluted site of the River Chenab at industrial and sewage waste disposal. The water sample was found viciously contaminated with heavy metals i.e. Ni, Cr, Mn, Co, Pb, Hg, Zn, Sn, Cu while all other physio-chemical variables crossed the suggested limits of WHO. The heavy metals load induced histopathological alterations were correlated to environmental degradation and the productivity of this biological system. *W. attu* and *C. mirigala* harvested from contaminated sites of the river indicated higher intensity of DNA damage through micronucleus induction and nuclear abnormalities with  $5.46 \pm 0.17$ ,  $1.23 \pm 0.08$  and  $4.2 \pm 0.11$ ,  $0.4 \pm 0.04\%$  respectively. Muscle sections of *W. attu* and *C. mirigala* harvested from the polluted section of river demonstrated the necrosis, degeneration of muscle fibers, intra-fibular edema and release of the blood into the tissues due to the bursting of blocked of the blood vessels. Dermal layers showed degeneration of the collagen bundles those were found loose or collapsed in some regions. Photomicrography also revealed vacuolar degeneration in muscle tissues and atrophy of muscle bundles. Intra fibular edema and splitting of muscle fibers were also seen along with bioaccumulation of toxicants. *W. attu* showed maximum incidence of alterations with highest histopathological alteration index related to environmental degradation. Control fish samples showed normal muscle tissues with normal equally spaced muscle bundles and myotomes.

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## 1. Introduction

Freshwater reservoirs receive most of the xenobiotics produced by anthropogenic activities. The bioaccumulation of the heavy metals may induce stress in aquatic fauna and flora and causing diseases of selected biota, enhanced lethality and extinction of the more sensitive taxa and disruption of the ecological balance (Padrihah et al., 2018). The increased pollutant load destroys aquatic fauna and flora in various trophic levels (Binelli and Provini,

2004). Physiological and biochemical parameters play important role as indicators of freshwater quality and to perceive the sub-lethal impacts of genotoxic compounds (Igwilo et al., 2006). Fish are excellent subjects to absorb, metabolize, concentrate and store such xenobiotics. Fish indicate the histopathological alterations, carcinogenic or mutagenic potential of such contaminants as in other animals (Al-Sabti and Metcalfe, 1995; Strzyzewska et al., 2016).

A simplest and quickest assay for genotoxicity assessment and biomonitoring is the micronucleus (MN) assay (Ali et al., 2008; Çavaş and Ergene-Gözükara, 2003) that put clear correlation to pollution load (Baršienė et al., 2013). Micronuclei are heterochromatin bodies formed by chromosomal fragments or chromosomes lag during anaphase failing to incorporate into daughter nuclei during cell division. Such chromosomal fragments by genetic damage results micronucleus formation and serves as an index of such damage (Ali et al., 2008). Fish also indicate morphological, cytological and histopathological changes in the different organs of the

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body in response to freshwater pollution (Ikram and Malik, 2009; Wahidulla and Rajamanickam, 2010; Deore and Wagh, 2012; Atli et al., 2015; Kaur et al., 2018). Heavy metals are directly associated with increasing incidence of cancer, neuromuscular damage, reproductive defects, and hyper susceptibility to variety of lethal diseases (Singla, 2015). This study was designed to determine whether freshwater pollution has genotoxic effects and histopathological alterations in *Wallago attu* (carnivorous) and *Cirrhinus mirigala* (herbivorous) occupying dissimilar niches.

## 2. Materials and methods

### 2.1. Water sampling

Five sampling locations were pre-determined along the River Chenab (31°34'14.0"N 72°32'02.8"E) upstream and downstream to Chakbandi Main Drain (CMD). Meroki (R1) and Thali (R2) were selected as upstream (control) while Maral Wala (R3), Binoi Said Jaial (R4) and Dhanu Wala (R5) were selected as downstream experimental sites. Seven water samples were collected from different points of each location to make a composite sample for physico-chemical parameters (Boyd, 1981) and Ni, Cr, Mn, Co, Pb, Hg, Zn, Tin and Cu. Metals were detected by "atomic absorption spectrophotometer" and heavy metal kits (Spectroquant® Merck) to achieve maximum accuracy level.

### 2.2. Fish samplings

Two fish species *Wallago attu* and *Cirrhinus mirigala* were collected from each site with seven fish samples of each species by using drag nets and gill nets in a weight range of 700–1100 g. Fresh Atria fish blood from a tail region vein was collected in heparin-coated tubes and used for micronucleus test. Dorsal-lateral body muscles from each fish specimen were used for histopathological studies.

### 2.3. Micronucleus assay

Fresh fish blood collected in heparin-coated tubes were "smeared on the slides, air dried" and fixation of the smear was performed in "cold Corney fixative for five minutes". Smeared "slides were stained in 10% Giemsa stain for thirty minutes (Hussain et al., 2016). Seven fishes of each fish species from each sampling site" and slides were prepared for each specimen totaling 35,000 count of erythrocytes/fish. "Presence of micronuclei in erythrocytes was detected and calculated under a Binocular fluorescent microscope (Nikon DS-fi2) Model Eclipse Ci-L under 40x and 60x magnifications" (Ali et al., 2008; Hussain et al., 2016). Erythrocytes with nuclear abnormalities (NAs) were calculated according to Bombail et al., 2001; Serrano-Garcia and Montero-Montoya, 2001; Cavas and Gözükar (2005) described by Obiakor et al. (2010).

### 2.4. Histopathology

Histopathological evaluations were performed by paraffin embedding method. Fish were dissected to remove the dorsal-lateral muscles. Tissues were sectioned and immediately fixed in 10% formalin to prevent autolysis (Ortiz et al., 2003). Tissues were then fixed in aqueous Bouin fixative for 24 h (Abalaka, 2017). Dehydration of fish muscle tissues was performed by isopropyl alcohol grading, washed with 50% ethanol, further dehydration was performed through 70%, 90% and absolute isopropyl alcohol and then cleared in xylene (Chavan and Muley, 2014). The tissues were processed and analyzed (Bernet et al., 1999; Ameer et al.,

2012; Deore and Wagh, 2012; Ortiz-Ordoñez et al., 2011; Chavan and Muley, 2014). Photomicrography of stained sections was performed under a microscope through 40x and 60x magnification ("Nikon DS- fi2 ECLIPSE Ci-L").

### 2.5. Statistical analysis

The data collected for water quality parameters was statistically by using SPSS 9 software. The means were compared by applying DMR tests ( $p < 0.05$ ). Regression analysis was performed on Microsoft excel 2010.

## 3. Results

Chakbandi Main Drain (CMD) plays an efficient role in polluting the River Chenab by draining urban and industrial sewage waste water of North-Eastern part of Faisalabad. The mean pH value of CMD was found ( $9.24 \pm 0.05$  mg/L) that is alkaline in nature. Water quality parameter assessment from the experimental sites downstream CMD showed high values of TSS, TDS, TS, Hardness, BOD, COD and conductivity even far higher than the suggested limits of WHO. Higher values of BOD, COD and salinity indicate organic pollution and salinity ( $85.09 \pm 1.11$  mg/L,  $135.57 \pm 1.94$  mg/L and  $1754 \pm 26.08$   $\mu$ S/cm) respectively. COD, pH, TS, Ni and Pb are found to be more responsible for the induction of MN and NAs. CMD also dispose higher levels of metallic salts into the River Chenab. The metals analyzed were nickel, Chromium, Manganese, Cobalt, Lead, Mercury, Zinc, Tin and Copper and were detected "higher than the admissible limits suggested by WHO" (Table 1).

DNA integrity through Micronuclei (MN) induction indicated significant DNA damage in the erythrocytes of the blood from both fish species. This assay showed that *Wallago attu* "harvested from contaminated site of the river (Table 2) downstream CMD displayed "highest frequency of micronucleus single" (MNs) (Figs. 1 and 2) and micronucleus double (MNd) (Fig. 3) with the mean values  $4.02 \pm 0.15$  and  $0.51 \pm 0.08\%$  cells, respectively, followed by *C. mirigala* with the mean values  $3.70 \pm 13.6\%$  cells (Figs. 4 and 5) and  $0.43 \pm 0.9\%$  cells (Fig. 6), respectively. In case of nuclear abnormalities (NAs) highest frequency showed by *C. mirigala* followed by *W. attu* with the mean values  $1.18 \pm 0.15\%$  cells (Fig. 7) and  $1.01 \pm 0.12\%$  cells (Fig. 8), respectively. Specimen of *W. attu* collected from upstream sites of CMD (control) showed a higher frequency of MNs, MNd and NAs (Fig. 9) compared to *C. mirigala* in quite normal ranges (Fig. 10) indicating the sensitivity of *W. attu*.

Correlation matrix for MNs, MNd and NAs in selected species from upstream and downstream sites of CMD exhibited highly significant ( $P < 0.01$ ) and a positive relationship with each other. MNs in *W. attu* exhibited highly significant ( $P < 0.01$ ) and a positive correlation to MNd in *W. attu*, NAs in *W. attu*, MNs in *C. mirigala*, MNd in *C. mirigala* and NAs in *C. mirigala* and vice versa. MNd in *W. attu* was positively and highly significantly correlated to NAs in *W. attu*, MNs in *C. mirigala*, MNd in *C. mirigala* and NAs in *C. mirigala*. Similar findings were noticed for NAs. Correlation of physicochemical parameters with MNs, MNd and NAs in both species from upstream and downstream to CMD exhibited highly significant with PHS with each other. MNs in *W. attu* was found PHS correlated with pH, BOD, COD, TS, Ni, Pb, and Sn While MNS in *C. mirigala* showed PHS correlation to pH, BOD, TSS, Ni, Co and Pb. MNd in *W. attu* was found PHS correlated to pH, BOD, COD, TS and Hg While MNd in *C. mirigala* showed PHS correlation to Pb and Zn only. NAs in *W. attu* was found PHS correlated to pH, BOD, COD, TS, Cr and Hg While NAs in *C. mirigala* showed PHS correlation to TSS, Pb, Hg and Cu. Other levels of significance are also shown in Table 3. MN induction and NAs in *W. attu* was found in the PHS correlated to MN induction and NAs in *C. mirigala* (Table 4).

**Table 1**  
Comparison of means (mean  $\pm$  SE) for water quality parameters of different sites from the River Chenab.

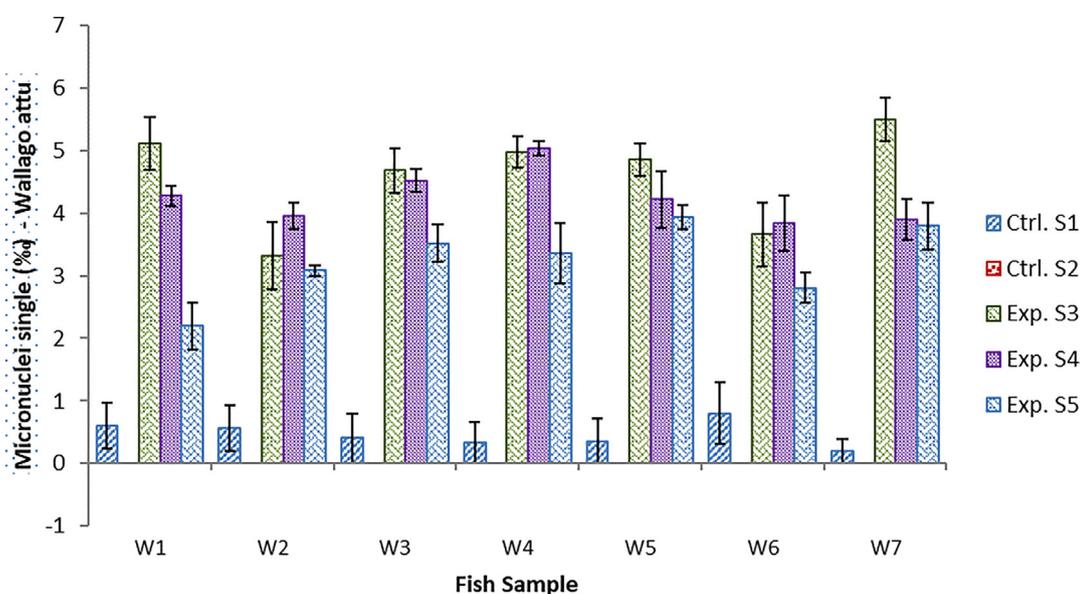
WQPs	“Site S1”	“Site S2”	“Site S3”	“Site S4”	“Site S5”
pH	7.50 $\pm$ 0.05 a	7.30 $\pm$ 0.08 a	9.69 $\pm$ 0.05 b	9.14 $\pm$ 0.04 b	8.90 $\pm$ 0.06 c
BOD (mg/L)	32.71 $\pm$ 1.06 a	37.29 $\pm$ 0.61 b	93.00 $\pm$ 0.93 c	84.14 $\pm$ 1.06 d	78.43 $\pm$ 1.36 e
COD (mg/L)	42.43 $\pm$ 0.65 a	38.00 $\pm$ 0.72 b	141.86 $\pm$ 1.84 c	135.57 $\pm$ 2.03 d	129.29 $\pm$ 1.96 e
Hardness (mg/L)	180.00 $\pm$ 5.35 b	205.71 $\pm$ 3.69 b	508.57 $\pm$ 4.04 d	548.57 $\pm$ 8.57 c	581.43 $\pm$ 11.84 c
Conductivity ( $\mu$ S/cm)	642.86 $\pm$ 17.1 b	828.57 $\pm$ 18.4 a	1157.14 $\pm$ 29.7 c	1742.86 $\pm$ 25.4 d	2364.29 $\pm$ 26.1 e
TSS (mg/L)	1.16 $\pm$ 0.034 b	1.05 $\pm$ 0.022 b	2.32 $\pm$ 0.05 d	1.85 $\pm$ 0.04 e	1.48 $\pm$ 0.041 c
TDS (mg/L)	1.05 $\pm$ 0.03 a	1.08 $\pm$ 0.02 a	1.84 $\pm$ 0.05 b	2.01 $\pm$ 0.07 c	2.05 $\pm$ 0.09 c
TS (mg/L)	2.20 $\pm$ 0.059 a	2.13 $\pm$ 0.049 a	4.15 $\pm$ 0.106 b	3.86 $\pm$ 0.11 d	3.53 $\pm$ 0.13 c
Ni (mg/L)	0.034 $\pm$ 0.003 a	0.066 $\pm$ 0.006 a	0.24 $\pm$ 0.01 b	0.21 $\pm$ 0.00 b	0.14 $\pm$ 0.00 d
Cr (mg/L)	0.04 $\pm$ 0.003 a	0.069 $\pm$ 0.006 a	0.283 $\pm$ 0.006 b	0.273 $\pm$ 0.003 b	0.206 $\pm$ 0.003 c
Mn (mg/L)	0.040 $\pm$ 0.002 a	0.136 $\pm$ 0.019 b	0.264 $\pm$ 0.004 c	0.189 $\pm$ 0.003 d	0.158 $\pm$ 0.002 e
Co (mg/L)	0.615 $\pm$ 0.021 a	0.721 $\pm$ 0.010 b	1.091 $\pm$ 0.005 c	1.029 $\pm$ 0.002 c	0.799 $\pm$ 0.002 e
Pb (mg/L)	0.074 $\pm$ 0.004 b	0.047 $\pm$ 0.004 b	0.86 $\pm$ 0.018 d	0.96 $\pm$ 0.009 d	1.65 $\pm$ 0.006 c
Hg (mg/L)	<0.001 $\pm$ 0.00 a	<0.001 $\pm$ 0.00 a	0.025 $\pm$ 0.001 c	1.04 $\pm$ 0.006 b	0.70 $\pm$ 0.003 d
Zn (mg/L)	0.039 $\pm$ 0.004 a	0.034 $\pm$ 0.004 a	1.05 $\pm$ 0.008 b	0.87 $\pm$ 0.020 c	0.91 $\pm$ 0.014 c
Sn (mg/L)	0.004 $\pm$ 0.001 a	0.003 $\pm$ 0.001 a	0.32 $\pm$ 0.003 b	0.24 $\pm$ 0.003 c	0.33 $\pm$ 0.010 b
Cu (mg/L)	0.05 $\pm$ 0.001 a	0.03 $\pm$ 0.002 a	0.91 $\pm$ 0.003 c	0.86 $\pm$ 0.003 b	0.83 $\pm$ 0.010 b

“Means sharing similar letter in a row belonging to particular parameter are statistically non-significant ( $P > 0.05$ ). COD; Chemical Oxygen demand, BOD; Biochemical Oxygen demand, TSS; Total suspended solids, TDS; Total dissolved solids, TS; Total solids. \* 31°34'14.0"N 72°32'02.8"E”.

**Table 2**  
Morphometric measurements of fish collected from River Chenab.

Variables	Head Length (cm)	Standard Length (cm)	Total Length (cm)	Wet Weight (g)	K
<i>Wallago attu</i>					
River Site S1	6.0 $\pm$ 1.02	44 $\pm$ 2.11	48 $\pm$ 2.641	1078 $\pm$ 67	1.2
River Site S2	5.6 $\pm$ 0.91	48 $\pm$ 2.09	53 $\pm$ 1.21	1119 $\pm$ 51	1.0
River Site S3	5.9 $\pm$ 0.89	52 $\pm$ 3.14	56 $\pm$ 2.01	1127 $\pm$ 83	0.8
River Site S4	6.7 $\pm$ 1.21	48 $\pm$ 6.75	52 $\pm$ 3.04	1200 $\pm$ 91	1.0
River Site S5	5.7 $\pm$ 0.96	47 $\pm$ 1.90	51 $\pm$ 2.02	989 $\pm$ 22	0.9
<i>Cirrhinus mrigala</i>					
River Site S1	6.5 $\pm$ 0.76	49 $\pm$ 3.29	54 $\pm$ 2.71	1017 $\pm$ 82	0.8
River Site S2	5.1 $\pm$ 0.82	48 $\pm$ 2.95	51 $\pm$ 2.61	1064 $\pm$ 99	0.9
River Site S3	6.5 $\pm$ 1.11	55 $\pm$ 3.64	58 $\pm$ 2.07	1189 $\pm$ 72	0.7
River Site S4	5.7 $\pm$ 0.98	49 $\pm$ 6.75	52 $\pm$ 2.04	1085 $\pm$ 96	0.9
River Site S5	6.1 $\pm$ 1.13	46 $\pm$ 2.91	47 $\pm$ 2.01	1006 $\pm$ 16	1.0

Sites S1 and S2 are control sites of the River Chenab; K; Fulton's condition factor.



**Fig. 1.** “Comparative analysis of single micronucleus induction (per thousand cells) in *Wallago attu* from different sites”.

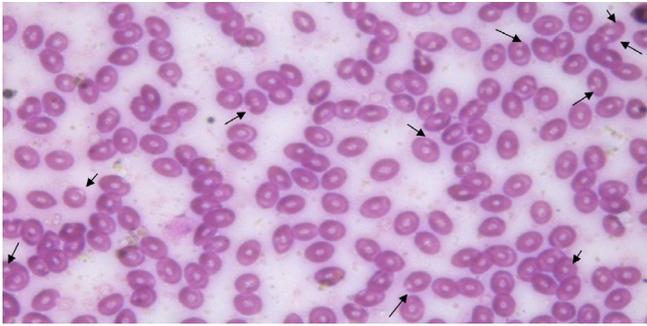


Fig. 2. Micronucleus assay of fish *Wallago attu* collected from polluted site of River Chenab indicating single micronucleus induction (Magnification 40x).

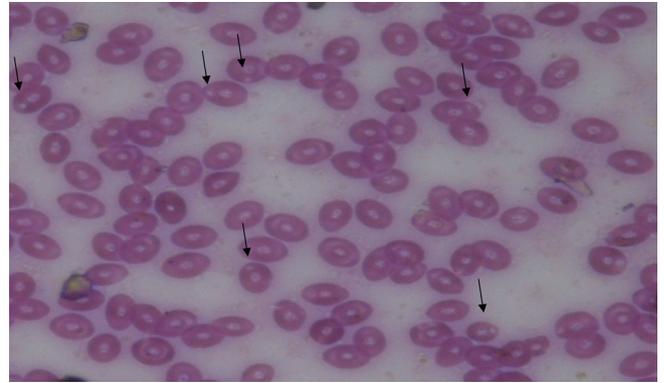


Fig. 5. Micronucleus assay for fish *C. mrigala* collected from polluted site of River Chenab indicating single micronucleus induction (Magnified 40x).

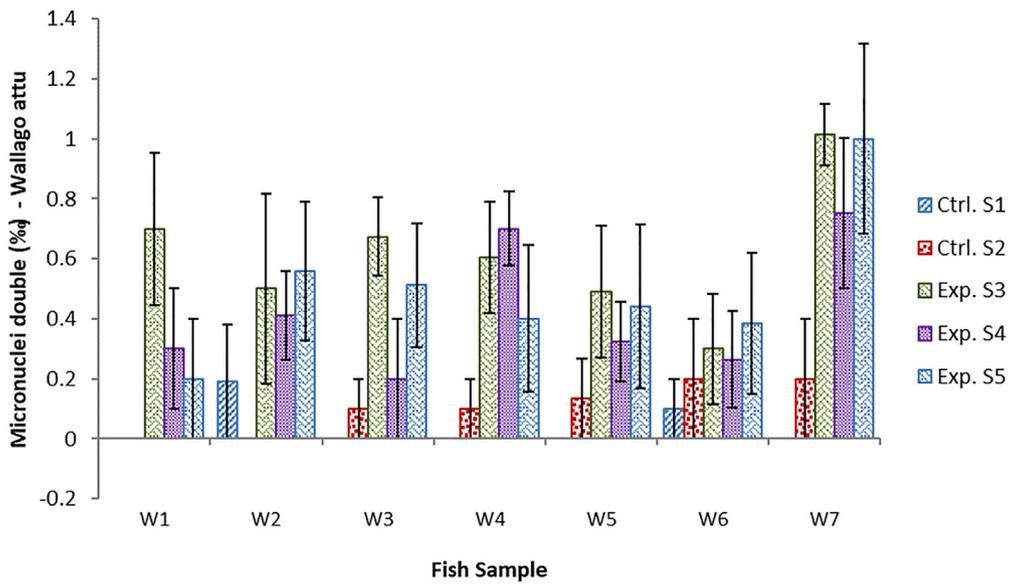


Fig. 3. Comparative analysis of double micronucleus induction (per thousand cells) in *Wallago attu* from different experimental sites.

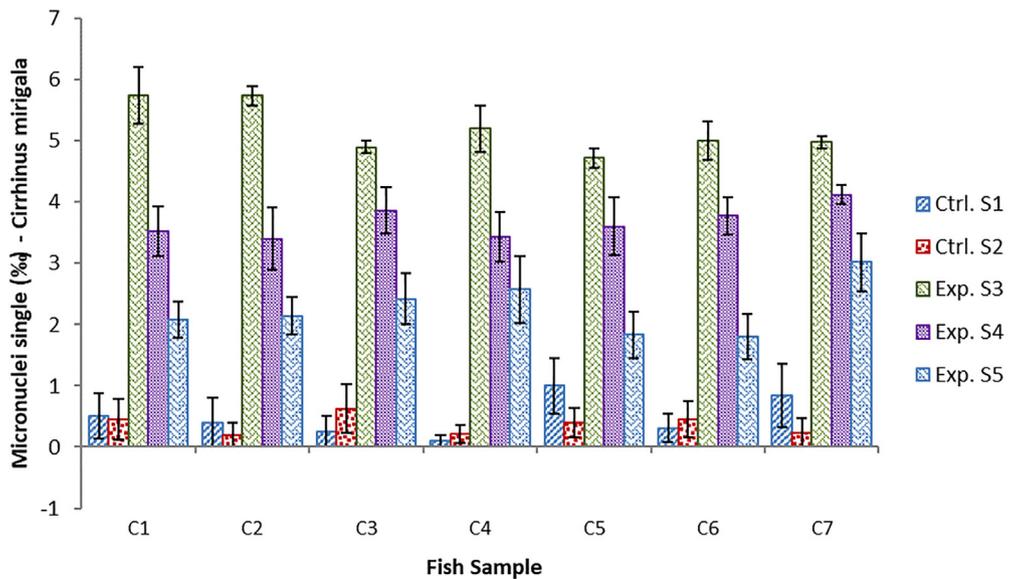


Fig. 4. Comparative analysis of single micronucleus induction (per thousand cells) in *C. mrigala* from different sampling locations.

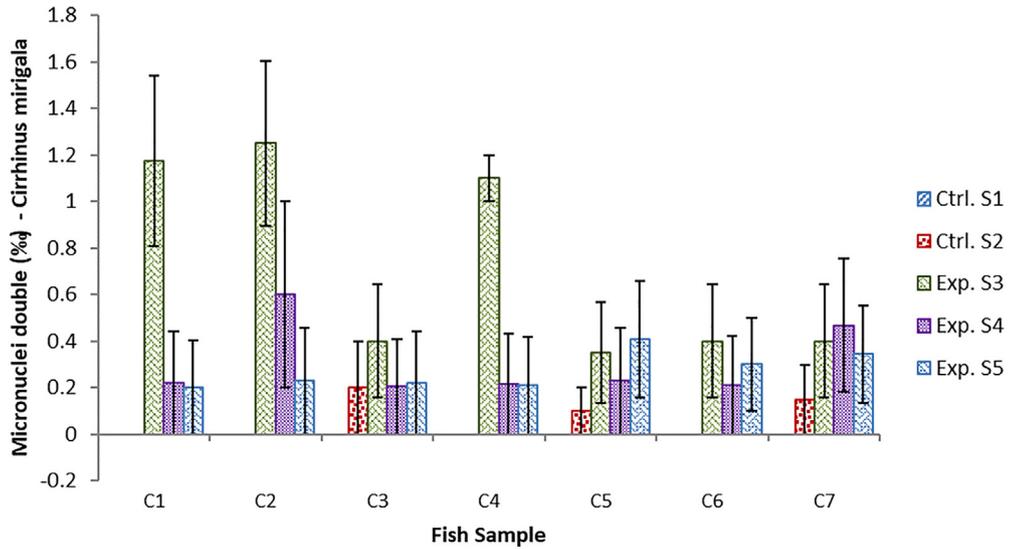


Fig. 6. Comparative analysis of double micronucleus induction (per thousand cells) in *Cirrhinus mirigala* from different sites.

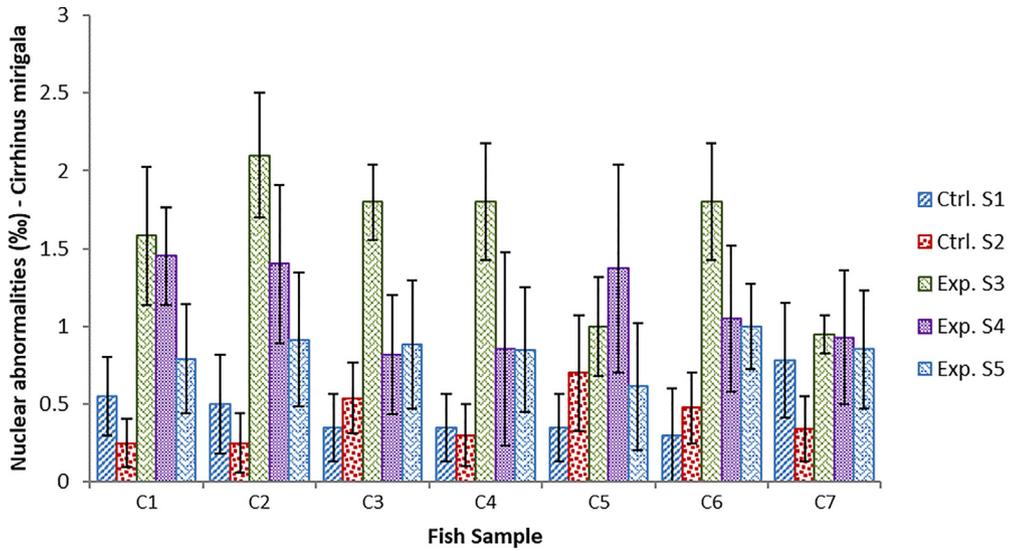


Fig. 7. Comparative analysis of nuclear abnormalities (%) in *Cirrhinus mirigala* from different experimental sites.

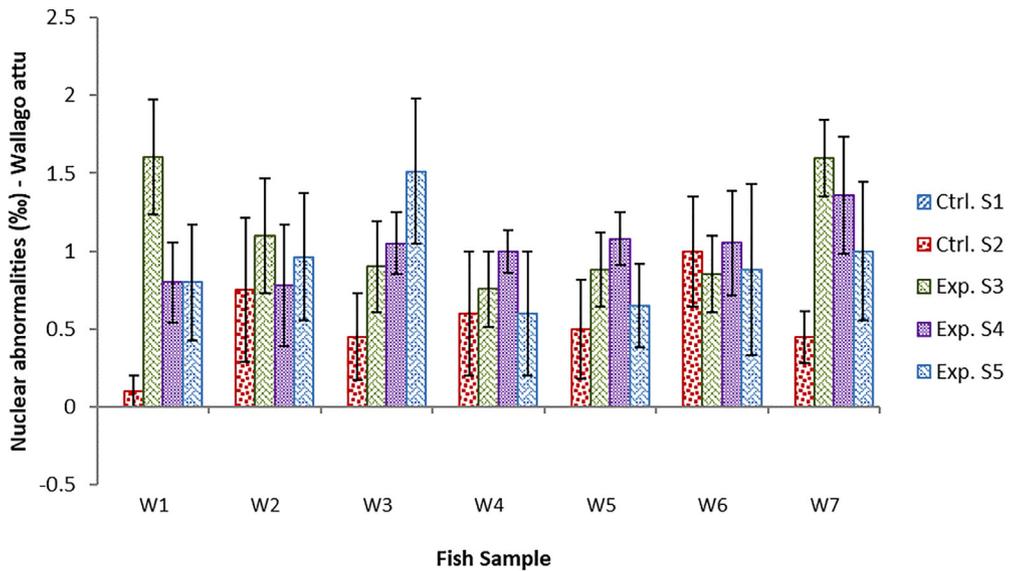
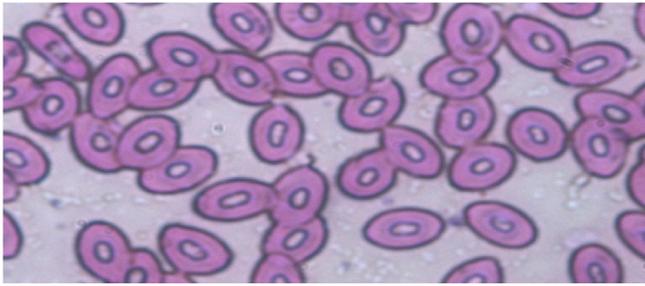
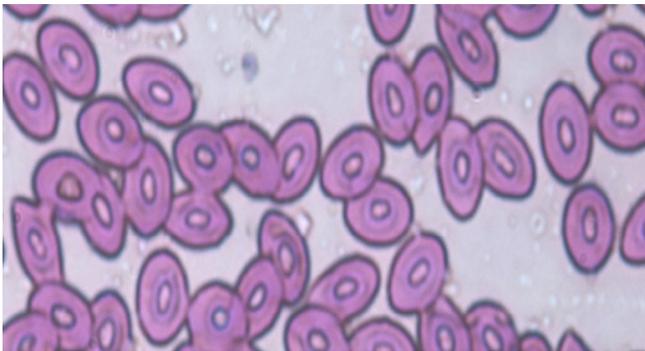


Fig. 8. Comparative analysis of nuclear abnormalities (per thousand cells) in *Wallago attu* from different site.



**Fig. 9.** Micronucleus assay for fish *Wallago attu* collected from upstream site to “entrance of Chakbani Main Drain into River Chenab indicating normal blood cells (Magnified 60x)”.



**Fig. 10.** Micronucleus assay for fish *Cirrhinus mrigala* collected from upstream site before entrance of “Chakbani Main Drain into River Chenab indicating normal blood cells (Magnified 60x)”.

Regression analysis also indicated” a unit increase in Ni, Cr, Mn, Co, Pb, Hg and all other physicochemical parameters MNs, MNd and NAs were increased in *W. attu* while in *C. mirigala*.

Normal skeletal muscle tissues are composed primarily of segmental myomeres. Each myomere is considered as apparent muscles and their fibers are parallel to the longitudinal axis of the organs or the main body. Photomicrograph for histopathology of flesh of *W. attu* harvested from the contaminated site of the river Chenab downstream CMD indicated the bioaccumulation of toxicants with significant indication of the necrosis. The flesh sections demonstrated the necrosis and degeneration of muscle fibers, intra-fibular edema and the release of the blood into the tissues due to the bursting of blocked blood vessels. Dermal layer showed degeneration of the collagen bundles that were loose in some regions and found collapsed in others. Micrographs also revealed “vacuolar degeneration in muscle tissues and atrophy of muscle bundles”. “Edema between muscle bundles and the splitting of muscle fibers” were also seen along with bioaccumulation of toxicants. Fish collected upstream CMD do not show any type of abnormality, but showed the normal architecture of muscle tissues and normal muscle fibers (Fig. 11).

Photomicrograph muscle tissues of *C. mirigala* harvested from the contaminated and experimental locations of the river downstream. CMD revealed the bioaccumulation of toxicants. Flesh micrographs demonstrated the degeneration of muscle fibers, intra fibular edema and release of the blood into the tissues. Photomicrograph also showed the degeneration of the collagen bundles in the tissues. Fish collected from less polluted site also revealed some type of abnormalities in the muscular tissues in the case of *C. mirigala* as slight loosening and aggregations of inflammatory cells in the muscle fibers (Fig. 12). Majority of control samples

**Table 3**  
Correlation among Physico-chemical parameters and MNs, MNd, NAs<sup>†</sup>.

	MNsW	MNdW	NAsW	MNsC	MNdC	NAsC
<b>pH</b>	0.990 <sup>**</sup> 0.001	0.969 <sup>**</sup> 0.006	0.992 <sup>**</sup> 0.001	0.966 <sup>**</sup> 0.007	0.904 <sup>*</sup> 0.035	0.957 <sup>*</sup> 0.011
<b>BOD</b>	0.992 <sup>**</sup> 0.001	0.980 <sup>**</sup> 0.003	0.970 <sup>**</sup> 0.006	0.941 <sup>*</sup> 0.017	0.875 0.052	0.925 <sup>*</sup> 0.025
<b>COD</b>	0.986 <sup>**</sup> 0.002	0.965 <sup>**</sup> 0.008	0.972 <sup>**</sup> 0.006	0.906 <sup>*</sup> 0.034	0.818 0.091	0.886 <sup>*</sup> 0.045
<b>Hard.</b>	0.929 <sup>*</sup> 0.022	0.931 <sup>*</sup> 0.022	0.894 <sup>*</sup> 0.041	0.792 0.110	0.696 0.192	0.763 0.134
<b>TSS</b>	0.924 <sup>*</sup> 0.025	0.875 0.052	0.943 <sup>*</sup> 0.016	0.994 <sup>**</sup> 0.001	0.958 <sup>*</sup> 0.010	0.997 <sup>**</sup> 0.000
<b>TDS</b>	0.925 <sup>*</sup> 0.024	0.914 <sup>*</sup> 0.030	0.890 <sup>*</sup> 0.043	0.780 0.120	0.670 0.216	0.748 0.146
<b>TS</b>	0.998 <sup>**</sup> 0.000	0.965 <sup>**</sup> 0.008	0.990 <sup>**</sup> 0.001	0.959 <sup>**</sup> 0.010	0.881 <sup>*</sup> 0.049	0.944 <sup>*</sup> 0.016
<b>Cond.</b>	0.624 0.261	0.673 0.213	0.567 0.319	0.383 0.524	0.284 0.644	0.343 0.572
<b>Ni</b>	0.976 <sup>**</sup> 0.004	0.923 <sup>*</sup> 0.025	0.948 <sup>*</sup> 0.014	0.977 <sup>**</sup> 0.004	0.906 <sup>*</sup> 0.034	0.961 <sup>**</sup> 0.009
<b>Cr</b>	0.993 <sup>**</sup> 0.001	0.945 <sup>*</sup> 0.015	0.962 <sup>**</sup> 0.009	0.948 <sup>*</sup> 0.014	0.858 0.063	0.926 <sup>*</sup> 0.024
<b>Mn</b>	0.849 0.069	0.879 <sup>*</sup> 0.050	0.793 0.110	0.895 <sup>*</sup> 0.040	0.924 <sup>*</sup> 0.025	0.893 <sup>*</sup> 0.041
<b>Co</b>	0.927 <sup>*</sup> 0.023	0.849 0.069	0.893 <sup>*</sup> 0.041	0.962 <sup>**</sup> 0.009	0.892 <sup>*</sup> 0.042	0.947 <sup>*</sup> 0.015
<b>Pb</b>	0.912 <sup>**</sup> 0.013	0.889 <sup>*</sup> 0.049	0.801 <sup>*</sup> 0.047	0.952 <sup>**</sup> 0.008	0.842 <sup>**</sup> 0.020	0.881 <sup>**</sup> 0.009
<b>Hg</b>	0.832 <sup>*</sup> 0.021	0.811 <sup>**</sup> 0.009	0.991 <sup>**</sup> 0.002	0.835 0.064	0.879 0.123	0.969 <sup>**</sup> 0.003
<b>Zn</b>	0.759 0.022	0.765 0.060	0.801 0.051	0.866 0.051	0.892 <sup>**</sup> 0.012	0.977 <sup>*</sup> 0.017
<b>Sn</b>	0.900 <sup>**</sup> 0.003	0.875 0.056	0.789 <sup>*</sup> 0.039	0.978 <sup>*</sup> 0.046	0.993 0.052	0.950 0.055
<b>Cu</b>	0.934 <sup>*</sup> 0.024	0.992 <sup>*</sup> 0.032	0.893 0.061	0.900 0.111	0.778 <sup>*</sup> 0.022	0.749 <sup>**</sup> 0.005

<sup>†</sup>Upper values indicated Pearson's correlation coefficient; Lower values indicated level of significance at 5% probability.

<sup>\*</sup> = Significant (P < 0.05).

<sup>\*\*</sup> = Highly significant (P < 0.01).

<sup>†</sup> = **MNsW**; (%) Micronuclei single in *W. attu*, **MNdW**; (%) Micronuclei double in *W. attu*, **NAsW**; (%) Nuclear abnormality in *W. attu*, **MNsC**; (%) Micronuclei single in *C. mirigala*, **MNdC**; (%) Micronuclei double in *C. mirigala*, **NAsC**; (%) Nuclear abnormality in *C. mirigala*”.

**Table 4**  
Correlation matrix for Micronucleus & nuclear abnormalities in fish *W. attu* and *C. mirigala*.

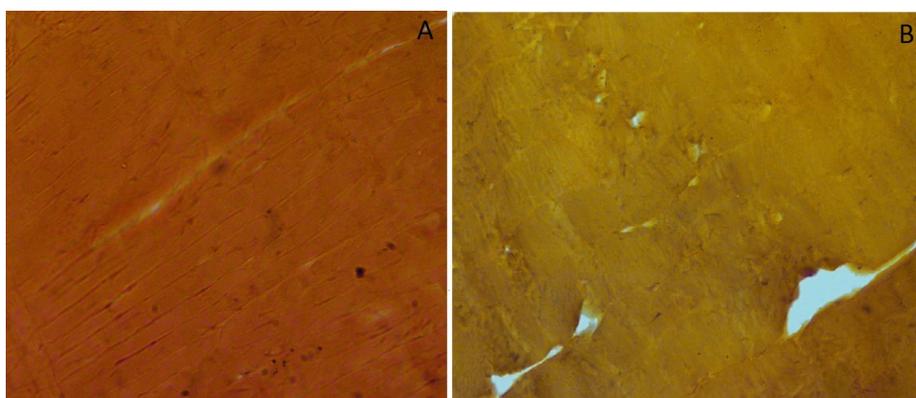
	MNsW	MNdW	NAW	MNsC	MNdC
<b>MNdW</b>	0.951*				
	0.013				
<b>NAW</b>	0.986**	0.933*			
	0.002	0.020			
<b>MNsC</b>	0.957*	0.911*	0.962**		
	0.011	0.032	0.009		
<b>MNdC</b>	0.864	0.900*	0.872	0.953*	
	0.059	0.037	0.054	0.012	
<b>NAC</b>	0.938*	0.905*	0.950*	0.997**	0.970**
	0.018	0.035	0.013	0.000	0.006

\*Upper values indicated Pearson's correlation coefficient; Lower values indicated level of significance at 5% probability.

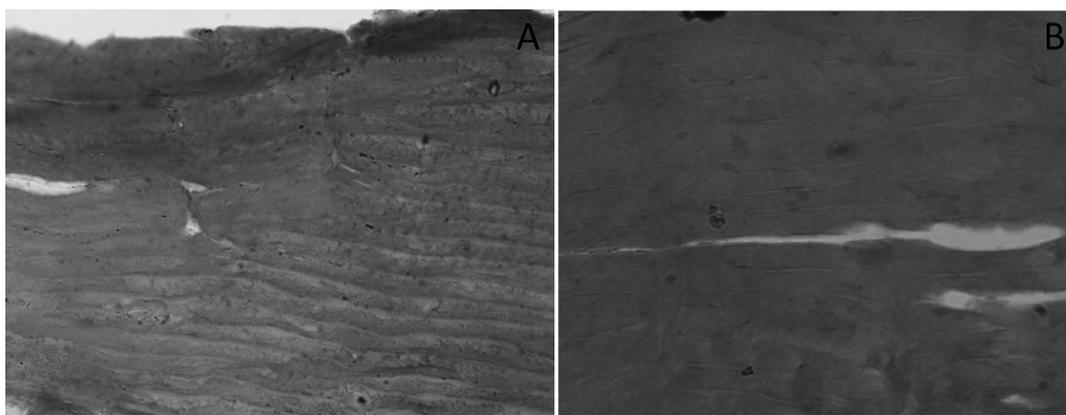
**MNsW;** (%) Micronuclei single in *W. attu*, **MNdW;** (%) Micronuclei double in *W. attu*, **NAW;** (%) Nuclear abnormality in *W. attu*, **MNsC;** (%) Micronuclei single in *C. mirigala*, **MNdC;** (%) Micronuclei double in *C. mirigala*, **NAC;** (%) Nuclear abnormality in *C. mirigala*.

\* = Significant ( $P < 0.05$ ).

\*\* = Highly significant ( $P < 0.01$ ).



**Fig. 11.** Photomicrograph of histological sections of muscles from *Wallago attu* collected from polluted river sites. (A): Normal muscle tissue of fish collected upstream CMD. (B): Defective muscle tissue of fish collected downstream CMD (40x10x magnification).



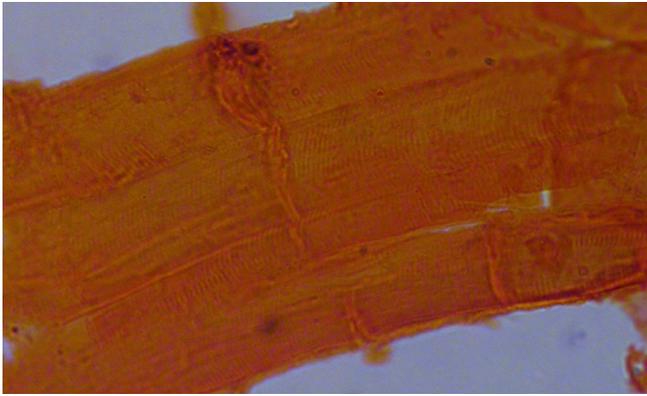
**Fig. 12.** Photomicrograph of histological sections of muscles from *Cirrhinus mirigala* collected from polluted river sites. (A): Defective muscle tissue of fish collected downstream CMD (B): Normal muscle tissue of fish collected upstream CMD. (40x10x magnification).

showed the normal muscle tissues, with normal equally spaced muscle bundles and normal myotomes when compared with farmed fish (Fig. 13).

#### 4. Discussion

The present study revealed that the presence of huge amount of heavy metals in the water has caused habitat destruction in the

River Chenab. This study found that untreated industrial and sewage waste water from Faisalabad has strongly polluted the water of "River Chenab" even not suitable for agriculture and riverine flora and fauna (El-Khayat et al., 2018; Kalaiyarasi et al., 2017). These toxicants present in water have the potential to cause chromosomal abnormalities and irreversible DNA damage, these findings strongly corroborate with the results of Alink et al. (2007) and Obiakor et al. (2010a). They mentioned induction of micronuclei and NAs during different exposure periods. "Fish exposed to pol-



**Fig. 13.** Photomicrograph of histological sections of muscles fibers of farmed *Cirrhinus mrigala* (60x10x magnification).

luted waters showed” a higher number of MN and NAs with comparison to control fish. [Obiakor et al. \(2010a\)](#) insisted the monitoring of the river “to prevent human genotoxicity through bioaccumulation and loss of aquatic biodiversity”. In world majority of industries release their waste, untreated, into Rivers so it also needs reduction in the use of unfriendly compounds and dyes, continuous monitoring, treatment of the wastes and proper disposal. [Ameur et al. \(2012\)](#); [Fu et al. \(2000\)](#); [Ramesh and Nagarajan \(2013\)](#) also reported the similar findings.

[De-lemos et al. \(2007\)](#) explained the quality of Cai River water by using MN assay in erythrocytes of Fathead minnow. The river was under the influence of petrochemical complex. Organisms exposed to contaminants were collected from four different sites. Exposed fish showed induction of MN and different NAs. This assay allowed the detection of genotoxicity and indicated the potential of monitoring for environmental genotoxicity ([Ansari et al., 2009](#); “[Wahidulla and Rajamanickam, 2010](#); [Seriani et al., 2012](#)”) also concluded that induction of MN frequencies is a very useful indicator and early warning for environmental genotoxicity. A study by [Braunbeck et al. \(2005\)](#); [Spitsbergen and Kent \(2003\)](#); [Osman et al. \(2007\)](#) indicated that the induction of MN depends upon the concentration of pollutants and while the time required to induce the MN and NAs decrease with increase in concentration of pollutants as the case here at the site R3 where CMD draining huge amounts of wastes. Both fish species *W. attu* and *C. mirigala* showed the maximum frequency of MNs, MNd and NAs. These findings also strongly matched with the findings of [Al-Sabti and Metcalfe \(1995\)](#); [Hayashi et al. \(1998\)](#); [Bombail et al. \(2001\)](#); [Ali et al. \(2008\)](#); [Ravindra et al. \(2010\)](#).

[De-Andrade et al. \(2004\)](#) also studied that agricultural runoff, municipal wastewater and industrial effluent having a large amount of complex substances disposed into the aquatic environment leading to the contamination of ground as well as surface water. They used MN assay to detect genotoxicity negotiating present study in case of River Chenab. They used blood from mullet and catfish and revealed the increased level of MN frequencies, NAs with reference to fish same as this project and related it to increased populations of the conurbation close to the sampling sites. These findings were also found to be similar to the findings of [Sun et al. \(2009\)](#); [Saleh \(2010\)](#); [Ameur et al. \(2012\)](#). [Arunachalam \(2013\)](#); [Ali et al. \(2008\)](#) determined that MN assay is a very sensitive indicator of aquatic pollution and declare it as a successful mutagenic assay. [Grisolia et al. \(2009\)](#) provided the fact that when all species belonging to particular environment were exposed to identical contaminants with their known feedings and habits, the piscivorous specimens showed a higher level of MN while herbivorous species showed lower frequency. They also

reported that fish is most appropriate animal to study the effect of toxicants as they are bioconcentrators ([Goksoyr et al., 1991](#); [Miracle and Ankley, 2005](#)). In this study model fish species *W. attu* and *C. mirigala* that are carnivorous and herbivorous fishes respectively, showed a similar type of findings. [Barbosa et al. \(2003\)](#) also found bioaccumulation and higher levels of metal salts in the tertiary consumers followed by omnivorous fish with regard to numerous toxicants accumulating along food chains and reach the uppermost concentration in top predator species ([Livingstone, 1993](#); [Caldas et al. \(1999\)](#); [Kelly et al. \(2007\)](#)).

The use of histopathological biomarkers “has been successfully employed” in fish to “assess the effect of pollutant on the” important vital organs in fish, which respond well to the toxicants as well as stress ([Abalaka, 2017](#)). [El-Khayat et al. \(2018\)](#) and [Reddy and Rawat \(2013\)](#) confirmed histopathology as priceless biomarkers for genotoxic assessments. They also pointed out that histopathological biomarkers have the ability to determine the effect of water pollutants, as observed in the current ([Peebua et al., 2008](#); [Jabeen and Chaudhry, 2010](#); [Reddy and Rawat, 2013](#); [Viana et al., 2013](#)). The present study revealed that *W. attu* and *C. mirigala* manifest histopathological changes in muscle and these pathological alterations in the muscles of all studied fishes. It could be a direct as well as the indirect indicator of the effect of genotoxicants, the heavy metals, pesticides, salts, industrial and domestic sewage wastes, entering into the river water by the network of drainage drains. These histopathological changes in the muscles were in line with the studies of [Mansour and Sidky \(2003\)](#); [Elnemaki and Abuzinadah \(2003\)](#); [Abbas and Ali \(2007\)](#); [Kaur et al. \(2018\)](#) reported the effect of various toxicants ([Chang et al., 2019](#)) on fish muscles, including the fish muscular destruction and vacillation when exposed to chromium whereas [Fatma \(2009\)](#); [Padrilah et al. \(2018\)](#) also revealed similar effects in the presence of Zn, Cu and Pb. Zn, Cu and Pb ([Reddy and Rawat, 2013](#); [Drishya et al., 2016](#); [Abalaka, 2017](#); [El-Khayat et al., 2018](#)).

## 5. Conclusions

*Wallago attu* and *Cirrhinus mrigala* procured from polluted sites indicated higher intensity of DNA damage through micronucleus induction and nuclear abnormalities. Muscle sections from “both the fish species from the” contaminated sites on the river demonstrated the necrosis, degeneration of muscle fibers, intra-fibular edema and release of the blood into the tissues due to the bursting of blocked of the blood vessels. Dermal layers showed degeneration of the collagen bundles those were found loose or collapsed in some regions.

## Declaration of Competing Interest

Authors have no conflict of interest.

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