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Effect of pollution on DNA damage and essential fatty acid profile in *Cirrhinus mrigala* from River Chenab*

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Abstract The objective of this study was to evaluate the effect of anthropogenic pollution on DNA damage and the fatty acid profile of the bottom dweller fish (*Cirrhinus mrigala*), collected from the River Chenab, in order to assess the effect of the toxicants on the quality of the fish meat. The levels of Cd, Hg, Cu, Mn, Zn, Pb, Cr and Sn and of phenols from this river were significantly higher than the permissible limits set by the USEPA. Comet assays showed DNA damage in *Cirrhinus mrigala* collected from three different sampling sites in the polluted area of the river. Significant differences were observed for DNA damage through comet assay in fish collected from polluted compared to control sites. No significant differences were observed for DNA damage between farmed and fish collected from upstream. The micronucleus assay showed similar trends. Fish from the highly polluted sites showed less number of fatty acids and more saturated fatty acids in their meat compared to fish from less polluted areas. Several fatty acids were missing in fish with higher levels of DNA in comet tail and micronucleus induction. Long-chain polyunsaturated fatty acids, eicosapentaenoic acid (20:5n-3) was found missing in the fish from polluted environment while it was found in considerable amount in farmed fish 7.8±0.4%. Docosahexaenoic acid (22:6n-3) also showed significant differences as 0.1±0.0 and 7.0±0.1% respectively, in wild polluted and farmed fishes.

Keyword: habitat; fish; comet assay; micronucleus assay; fatty acids

1 INTRODUCTION

Fish are considered to be one of the most important sources of animal proteins. During the recent past, the potential of fish culture has led to its large-scale adoption and promotion in Asian countries. Also in recent years, "lipids from fish meat have assumed a great nutritional significance owing to their protective role against the development of cardiovascular diseases and rheumatoid arthritis (Ackman, 1967; Burr et al., 1989; Polvi and Ackman, 1992; Shahidi and Boota, 1994). Coronary heart diseases have been identified as a leading cause of death in various parts of the world, including Pakistan, with mortality rates increasing every year (Kiessling et al., 2001; Kandemir and Polat, 2007)".

Recently, however, concern about aquatic environmental pollution has increased, especially in respect to aquatic water bodies such as rivers. Municipal wastewater comprises 99.9% water with small concentrations of dissolved and suspended organic and inorganic solids. Among organic substances, there are synthetic detergents, soaps, fats, proteins, carbohydrates, lignin and their decomposition products. Natural and synthetic (organic and inorganic) chemicals also add major toxic compounds from industry. These types of water pollution directly and indirectly influence aquatic life by modifying genomes (Villela et al., 2006; Nhapi et al., 2011). The effects of such pollution have combined with overfishing to meet increasing demand to lead to a drastic decrease in fish populations.

Contaminated water from industrial and

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metropolitan areas of Faisalabad, Pakistan is discharged of into the River Chenab, which has forced to the extinction, or reduction in population, of Indian major carps along almost 190 km of the River Chenab. Much of this industrial and sewage waste enters the River Chenab from the eastern and southern parts of Faisalabad through the Chakbandi Drain. This polluted water contains large amounts of toxic chemicals from a variety of industries such as the textile, chemical and pharmaceutical industries, and tanneries and sugar mills.

Mrigal (*Cirrhinus mrigala*), a carp endemic to sub-continent riverine systems, is one of the three Indian major carp species cultivated widely in India, Pakistan, Burma and Bangladesh. Mrigal has long been important in polyculture practices with other indigenous fish species in the country. The initially higher growth rate of mrigal, coupled with its compatibility with other carps, has helped in establishing this species as one of the principal component species in pond culture. The compatibility of *C. mrigala* in polyculture systems with other carps has already been documented. Mrigal was selected for this unique study considering its popularity among the consumers and economic importance. The present study was conducted to assess the effect of water pollution on DNA damage and the fatty acid profile of fish in the river Chenab in order to show that fish in that river not only bioaccumulate toxicants in their body but also exhibit reduced meat quality.

2 MATERIAL AND METHOD

2.1 Study area

The Chenab River is a major river of India and Pakistan. It forms in the upper Himalayas in the Lahaul and Spiti district of Himachal Pradesh, India, and flows through the Jammu region of Jammu and Kashmir into the plains of the Punjab, Pakistan. Sewage and industrial waste from Faisalabad city are disposed of into the River Chenab through the Chakbandi drain at latitude 31.570°N and longitude 72.534°E (Fig.1).

Fish were harvested from the river in Pakistan, through 190 km upstream of Trimu Head to Thalli. Three sites viz. Wara Thatta Muhammad Shah (R1), Bela Reta (R2) and Bandimahni Beg (R3) were selected, all being exposed, to different extents, to polluted water from Chakbandi Drain. Two sites, Libhan Wala (U1) and Thali (U2), were selected as upstream sites with unpolluted water (i.e. before exposure to the Chakbandi Drain to allow comparison of wild fish. "Apart from during the rainy season, only polluted water from drains flows through the River Chenab up to Trimu Head. Drag nets and gill nets were used to harvest fish from these highly polluted areas (R1, R2 and R3) of the river, as well as upstream of this area." Farmed fish (negative control) of required weight category was collected from the ponds of two different sites of Satiana Road fish hatchery Faisalabad, where one was exposed to pollutants (salts of heavy metals, polycyclic aromatic hydrocarbons and mixture of textile dyes etc.) and was designated as positive control. Fish was harvested by drag nets in the weight range of 500–880 g. Seven fish samples were collected from each sampling site and each sample was analysed thrice.

2.2 Preparation of fish samples

All the fish specimens were transported in polythene bags to the Fisheries Laboratory, Zoology, Government College University Faisalabad, and prepared by following the method described by Mahboob et al. (2014). The muscles of the fish samples were then washed with distilled water and cut into small pieces (2–3 cm) with a knife. Then, the muscle tissue was oven dried at 65°C until it reached a constant weight and dried samples were powdered using a glass mortar, sieved through 1 mm mesh and stored in airtight plastic vials inside desiccators.

2.3 Water analysis

Water samples were taken from the river at every point during summer and winter season from which fish were harvested and these were then analyzed for selected heavy metals and other water quality parameters, as defined by the Environmental Protection Agency of Pakistan and by Boyd (1981). The selected heavy metals analyzed were tin (Sb), chromium (Cr), lead (Pb), zinc (Zn), manganese (Mn), cupper (Cu), cadmium (Cd) and mercury (Hg). The concentration of each metal was detected by using heavy metal kits and Hitachi polarized Zeeman Atomic Absorption Spectrophotometer AAS, 2000 series. The blanks and calibration standard solution were also analyzed in the same way as for the samples. The instrument calibration standards were set using a diluting standard ($1\ 000\times10^{-6}$) supplied by Merck, Germany. A known 1 000 mg/L concentration of all the above mentioned metal solutions was prepared from their salts. All reagents used were of analytical grade. The percent recoveries in all the cases were

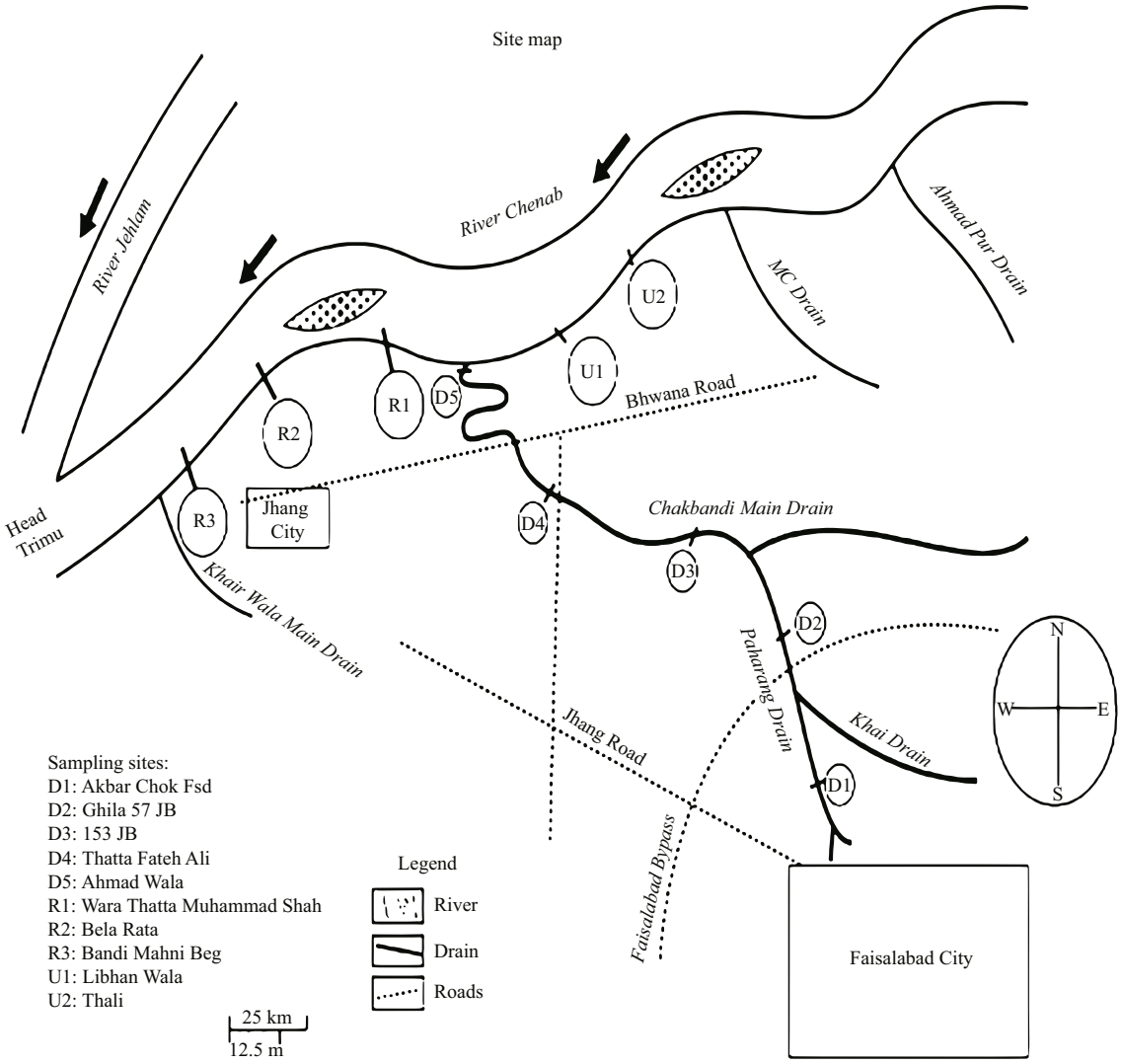

Fig.1 Site map of the study area indicating experimental

within the acceptable limits of 70% to 120%, as per regulatory guidelines.

2.4 Fatty acid profiling

Lipid contents were extracted from the powdered meat by a Soxhlet extraction unit with n-Hexane at 65°C. Extracted lipids were converted to fatty acid (FA) methyl esters using methanolic sulphuric acid through an esterification process (Tocher et al., 2003). The samples in a miniature vial were once again supplied with N_2 gas to remove ether and then were injected into the Gas Chromatograph. The sample injected was 0.2 µL with carrier gases N_2, H_2 and air 30, 40 and 500 mL/min respectively, column length 2m, column packed with 10% Diethyl glycol succinate with diameter of 2 mm. Column temperature was set at 190°C, injection port temperature 200°C and detection port temperature 250°C. The chromatograms produced from each sample were used to determine the retention time of each fatty acid (Fatty acid methyl esters (FAMEs)) and these were compared to the chromatogram of a standard (mixture of pure FAMEs). Fatty acids were determined by gas-liquid chromatography and flame ionization detection (Hedayatifard and Jamali, 2008).

2.5 DNA damage

"Two µL of fresh venous blood collected from caudal vein of freshly harvested fish was suspended in 0.5% low melting agarose on frosted microscopic slides. Another layer of 0.6% normal melting agarose was added on the top of first layer to sandwich blood cells in between these two agrose layers. The slides were placed on the ice for polymerization of the gel. After agarose solidification, slides were immersed in lysis buffer (2.5 mol/L NaCl, 1% sodium sarcosinate,

Table 1 Comparison of means of water quality parameters in the River Chenab (mean±SE)

Sites	Cadmium (mg/L)	Copper (mg/L)	Manganese (mg/L)	Zinc (mg/L)
R1	0.139±0.012^{ab}	0.907±0.212^{b}	**01.59±0.150^{c}**	**00.215±0.036^{b}**
R2	0.135±0.013^{b}	0.863±0.211^{b}	**01.53±0.138^{c}**	**00.207±0.036^{b}**
R3	0.130±0.014^{b}	0.826±0.203^{c}	**01.36±0.139^{b}**	**00.206±0.035^{a}**
Sites	Lead (mg/L)	Chromium (mg/L)	Tin (mg/L)	Mercury (mg/L)
R1	1.501±0.151^{c}	0.349±0.051^{a}	0.304±0.037^{b}	**00.996±0.033^{b}**
R2	1.348±0.120^{c}	0.289±0.040^{b}	0.273±0.030^{a}	**01.013±0.017^{c}**
R3	1.298±0.121^{a}	0.246±0.032^{a}	0.261±0.030^{a}	**00.893±0.012^{c}**
Sites	Phenols (mg/L)	Sulfates (mg/L)	BOD (mg/L)	COD (mg/L)
R1	**01.67±0.145^{a}**	264.79±47.23^{a}	70.64±2.33^{b}	146.43±13.61^{a}
R2	**01.48±0.121^{b}**	250.36±47.27^{b}	61.70±1.88^{c}	135.00±13.40^{c}
R3	**01.32±0.135^{c}**	246.07±45.68^{b}	50.88±1.44^{a}	124.07±13.87^{b}
Sites	pH	TDS (mg/L)	Salinity	Conductivity (mS/m)
R1	10.37±0.053^{a}	1 597.64±221.95^{c}	1 392.86±153.16^{c}	**02.25±0.258^{a}**
R2	10.28±0.019^{a}	1 475.43±220.16^{b}	1 250.00±145.16^{a}	**02.11±0.269^{a}**
R3	10.06±0.044^{b}	1 214.43±237.61^{a}	921.43±137.15^{b}	**01.70±0.309^{c}**

Means sharing the same letter in a row or in a column are statistically non-significant (P>0.05). R1–3: polluted experimental sites in the river; BOD: biochemical oxygen demand; COD: chemical oxygen demand.

10 mmol/L Tris-HCl, 100 mmol/L Na2EDTA, 1% Triton X-100 and 10% Dimethyl Sulphoxide) at 4°C. After 1 h of lysis slides were placed in the electrophoresis buffer (0.3 mol/L NaOH, 1 mmol/L Na2EDTA, pH 10) to facilitate unwinding of DNA in the cells. After 20 min slides were processed for electrophoresis (300 mA and 20 V for 30 min) and followed by neutralization by Tris-HCl buffer at 25°C. Visualization of cells and the DNA was accomplished by fluorescent microscopy after staining slides with 10% ethidium bromide following the protocol adapted by Dhawan et al. (2009). In micronucleus assay, fresh blood was smeared on a clean microscopic slide and left to air-dry at 25°C for 2 h in a moisture and dust free environment. Fresh blood smear was air dried and then fixed in cold Corney's fixative for 5 min. The slides were then fixed in methanol for 10 min and let them to air dry 25°C for 1 h. The slides were stained in 10% aqueous Giemsa for 30 min. The slides were then washed thoroughly in double distilled water and air dried. Seven fish were analyzed for a total 35 000 erythrocytes/fish sample in replica of five. The frequencies of micronuclei in erythrocytes were

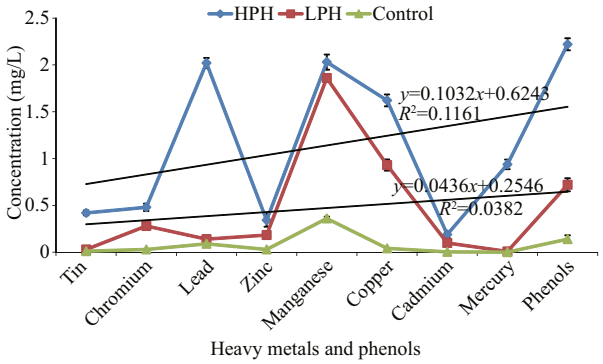

Fig.2 Concentrations of selected metals in river water and fish farm water

HPH: highly polluted environment; LPH: less polluted environment (upstream); control: fish farm.

scored under a Binocular microscope at T1200× magnification. Erythrocytes with intact cellular and nuclear membranes were also scored (Alink et al., 2007; Obiakor et al., 2010)".

2.6 Statistical analysis

The mean, standard error and analyses of variance (ANOVA) were calculated using SPSS-10 for PC. The means were compared by using Duncan's Multiple Range test. Probability values of P<0.05 were considered significant. Quantification of the DNA damage was performed with TriTek Cometscore freeware 1.6.1.13 (TriTek Corporation Summerduck, VA).

3 RESULT AND DISCUSSION

The water quality parameters analyzed showed an acute level of pollution load in the River Chenab (Table 1 and Fig.2). For water samples collected in summer heavy metal levels were "Cd (0.147±0.002 mg/L), Cu (0.881±0.00 mg/L), Mn (1.92±0.01 mg/L), Zn (0.168±0.01 mg/L), Pb (1.647±0.00 mg/L), Cr (0.256±0.006 mg/L), Sn (0.334±0.01 mg/L) and Hg (1.158±0.06 mg/L). In winter, however, the levels were recorded as Cd (0.218±0.005 mg/L), Cu (1.974±0.02 mg/L), Mn (2.65±0.06 mg/L), Zn (0.488±0.002 mg/L), Pb (2.956±0.003 mg/L), Cr (0.644±0.004 mg/L), Sn (0.466±0.011 mg/L), Hg (1.412±0.013 mg/L"). The higher values of heavy metals observed in winter may be due to the relatively lack of dilution of the river water in winter, since during these months only drain water flows through the length of the river. Water dilution occurs during the summer months due to the rains and the glacial meltwater. In both summer and

Table 2 DNA damage in the blood cells of *Cirrhinus mrigala* analysed by Comet Assay

Experimental Sites	DNA damage analyzed by Comet Assay				
	*DNA in tail (%)	Tail length (px)	Head diameter (px)	Tail moment	Olive moment
			Species, polluted site and type interaction (means±SE)		
R1.+ve	31.0±2.8^{ac}	65.0±6.2^{a}	148.0±10.7^{ab}	23.2±3.5^{a}	16.5±1.9^{a}
R1. F	3.8±0.6^{g}	1.3±0.3^{g}	51.9±1.6^{d}	0.1±0.0b^{f}	0.6±0.1b^{c}
R1. P	37.3±2.5^{a}	50.3±5.7^{ab}	104.0±4.7^{ce}	23.5±3.9^{a}	16.2±2.0^{a}
R1. U	13.2±2.2$^{d–g}$	9.0±1.7f^{g}	55.9±4.3^{bd}	1.9±0.4^{f}	2.1±0.4f^{g}
R2.+ve	15.6±2.5$^{d–g}$	6.4±1.2^{c}	37.4±2.8^{b}	2.0±0.6^{g}	2.6±0.4$^{c–g}$
R2. F	4.3±1.1^{fg}	1.6±0.4^{g}	52.8±2.1^{cd}	0.3±0.1^{f}	0.8±0.2^{g}
R2. P	35.0±2.5^{a}	43.4±6.6^{c}	89.4±6.5^{f}	19.8±4.3^{ag}	13.8±2.0^{ab}
R2. U	10.7±1.8$^{d–g}$	37.8±5.8$^{d–g}$	129.1±7.2^{bd}	8.5±2.3$^{c–f}$	7.1±1.4$^{c–g}$
R3.+ve	7.0±1.0$^{f–g}$	35.1±6.3^{ab}	141.7±4.8^{ab}	4.1±1.1^{ef}	4.7±0.9^{ae}
R3. F	8.2±2.5^{ef}	3.7±0.98^{fg}	52.3±1.9^{d}	1.3±0.6^{g}	2.2±0.9^{f}
R3. P	38.8±2.4^{a}	30.5±3.13^{d}	71.5±3.4^{fg}	14.3±1.8^{ac}	11.0±0.9$^{a–d}$
R3. U	33.1±2.7^{ab}	54.6±5.19^{ab}	98.1±4.9^{e}	22.1±3.0^{bf}	16.1±1.6^{b}
			Species and type interaction (means±SE)		
Control+ve	18.0±1.5^{bcd}	36.0±3.5^{ab}	109.0±5.7^{b}	10.0±1.5^{bc}	8.0±0.9^{bc}
Farmed	5.4±0.9^{e}	2.2±0.4^{e}	52.3±1.1^{e}	0.6±0.2^{d}	1.2±0.3^{d}
Polluted	36.8±1.4^{a}	41.5±3.1^{a}	87.8±3.9^{cd}	20.0±2.0^{a}	13.7±1.0^{a}
Upstream	19.0±1.5^{bc}	33.8±3.1^{ab}	94.5±4.0^{cd}	11.0±1.4^{b}	9.0±0.9^{b}
			Species and polluted site interaction (means±SE)		
R1	21.2±1.45^{a}	31.4±2.9^{a}	89.9±4.2^{a}	12.2±1.5^{a}	8.9±0.9^{a}
R2	16.4±1.31^{b}	22.3±2.6^{b}	77.2±3.6^{b}	7.6±1.3^{c}	6.1±0.7^{b}
R3	21.7±1.51^{a}	31.0±2.5^{a}	90.9±3.1^{a}	10.4±1.1^{b}	8.5±0.78^{a}
Means	19.8±0.83^{A}	28.21±1.5^{A}	6.0±2.1^{B}	10.1±0.8^{A}	7.8±0.4^{A}

Means sharing a similar letter in a column are statistically non-significant (P>0.05). Lowercase letters represent comparison among interaction means and uppercase letters are used for overall means. * Migrated DNA from the core of the cell (major indicator of DNA damage). Px: pixels, R1–R3: polluted experimental sites in the River; fish types (F: farmed; P: polluted; U: upstream; +ve; positive control).

winter, however, concentrations of these heavy metals were substantially higher than the USEPA (1999) permissible limits (Pb 0.002 5, Cd 0.02, Cr 0.011, Zn 0.12 and Cu 0.009 mg/L) and were more than enough to have adverse effects on fish health.

The maximum level of DNA damage was recorded in *C. mrigala* collected from site R3 followed by sites R1 and R2, respectively (Table 2). There was a significant difference (P<0.05) in DNA damage in *Cirrhinus mrigala* collected from site R1 between each of positive control, farmed and polluted fish (148.0±10.7, 51.9±1.65 and 104.0±4.7 respectively), and between polluted and upstream fish (104.0±4.66 and 55.9±4.35 respectively). For site R2 significant differences were obtained between farmed, polluted and upstream site samples (52.8±2.1, 89.4±6.5 and 129.1±7.2, respectively). For site R3 significant differences were obtained between positive control, polluted and upstream fish (141.7±4.8, 71.5±3.4 and 98.08±4.84) but non-significant results were found between farmed and polluted fish samples (52.3±1.9 and 71.5±3.4). The maximum tail moment was observed in the fish samples collected from R1, closely followed by R3. Fish from the site R2 showed non-significant (P>0.05) differences for all sites—positive control, farmed, polluted and upstream. Significant differences were reported, however, between farmed and polluted area (R3) fish. In respect to the olive moment of *C. mrigala*, those from R1 exhibited significant (P<0.05) differences with farmed fish and fish collected from the R3. *C. mrigala* showed the highest value for the head diameter in samples from site R1. Differences in comet tail length (px) in fish specimens collected from different sites were found to be highly significant (P<0.01). DNA damage assessed by micronucleus assay also exhibited

Table 3 DNA damage by Micronucleus assay of *Cirrhinus mrigala* blood

Fish type	Micronucleus assay components		
	Single micronucleus	Double micronucleus	Nuclear abnormalities
Polluted	44.8±3.7^{a}	6.2±1.0^{ab}	9.6±1.7^{a-d}
Upstream	20.6±4.0^{bcd}	5.2±1.5^{b}	10.0±1.0^{a-d}
Control (farmed)	8.2±2.2^{cd}	0.8±0.4^{b}	6.2±1.8^{bcd}
Positive control	37.4±3.9^{ab}	8.4±2.8^{ab}	15.2±2.1^{ab}
Means	27.7±3.7^{A}	5.1±1.0^{AB}	10.2±1.1^{A}

Means sharing a similar letter in a column are statistically non-significant (P>0.05). Small letters represent a comparison among interaction means and capital letters are used for the overall mean. Frequency calculated per thousand cells.

significant differences between fish collected from polluted and upstream sites (Table 3). Fish from R2 and R3 showed significant differences with farmed fish but non-significant differences were recorded between fish samples collected from control and upstream sites (Table 3). In the case of % DNA in the tail significant (P<0.05) differences were reported between farmed and polluted area fish and also between polluted and upstream sites, but non-significant differences were reported between fish collected from a commercial fish farm (control) and upstream (U2). The control and upstream sites and exhibited non-significant (P>0.05) differences in pollution load. Highly polluted sampling sites (R3) exhibited significant differences with less polluted sites upstream (U2). The U2 site, however, differs significantly from the control sites and significant differences were observed between control, R3 and U2 sampling sites.

Fish collected from the polluted area of the River Chenab showed the highest frequency of micronucleus induction and nuclear abnormalities, therefore. *C*. *mrigala*, however, showed a considerable amount of micronucleus induction even when collected from lower pollution intensity areas (upstream to the entrance of Chakbandi Drain) (Table 3). Perhaps this is because *C*. *mrigala* is a bottom feeder and has maximum exposure to the polluted sediments. Farmed (control) fish showed a negligible amount of this kind of DNA damage.

The detected variation in individual fatty acids of the total lipid portion are shown in Table 4. The results shown for fatty acids of total lipids were limited to fish meat of two different ecological niches. One was highly polluted sites, where industrial and household

Table 4 Devastation and variations in the major classes of essential unsaturated fatty acids in *Cirrhinus mrigala* meat on the basis of polluted environment

Fatty acid	Farmed fish (mean±SE)	Upstream area fish (mean±SE)	Polluted area fish (mean±SE)
C18:3 (n-3)	1.3±0.02^{a}	0.8±0.0^{b}	0.5±0.02^{c}
C18:4 (n-3)	0.1±0.0^{b}	0.08±0.02^{b}	-
C20:5 (n-3)	1.6±0.08^{b}	0.6±0.04^{c}	-
C22:5 (n-3)	0.4±0.02^{a}	-	0.7±0.0^{b}
C22:6 (n-3)	1.4±0.04^{a}	1.4±0.0^{b}	0.02±0.0^{c}
C18:2 (n-6)	0.002±0.0^{a}	0.2±0.02^{a}	-
C20:2 (n-6)	0.1±0.0^{bc}	-	0.2±0.02^{a}
C20:4 (n-6)	2.1±0.14^{c}	0.9±0.0^{a}	1.6±0.0^{b}
C20:5 (n-6)	0.02±0.0^{a}	1.5±0.02^{b}	0.6±0.02^{c}
C22:4 (n-6)	0.08±0.0^{a}	-	-
C22:5 (n-6)	1.2±0.02^{c}	0.6±0.02^{a}	0.02±0.0^{b}
C16:1 (n-7)	0.1±0.0^{a}	-	-
C18:1 (n-7)	0.3±0.0^{a}	0.06±0.0^{b}	0.1±0.02^{a}
C16:1 (n-9)	0.5±0.0^{ac}	0.08±0.0^{b}	-
C18:1 (n-9)	2.4±0.02^{b}	0.02±0.0^{c}	-
C20:1 (n-9)	0.7±0.04^{a}	-	-
C22:1 (n-9)	0.12±0.0^{b}	0.7±0.02^{b}	1.0±0.02^{c}
Σn-3 PUFA	4.7±0.06^{a}	2.9±0.02^{b}	1.2±0.02^{c}
Σn-6 PUFA	3.5±0.08^{b}	3.2±0.02^{b}	2.4±0.01^{c}
Σn-7 MUFA	0.4±0.0^{a}	0.06±0.0^{a}	0.1±0.02^{b}
Σn-9 MUFA	3.7±0.04^{a}	0.1±0.02^{c}	1.0±0.01^{b}
ΣPUFA	8.3±0.18^{b}	6.5±0.02^{b}	3.6±0.02^{b}
ΣMUFA	4.1±0.04^{a}	0.2±0.02^{b}	1.2±0.02^{c}
ΣUFA	12.4±0.28^{a}	6.3±0.02^{c}	4.8±0.02^{b}
ΣSFA	7.6±0.58^{a}	13.7±0.76^{b}	15.2±0.88^{c}

Data are mean expressed as a g/100 g of dry fish meat from seven fish samples/site analysed in triplicate. Different letters in the same row represent significant (P<0.05) difference. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; MUFA: polyunsaturated fatty acids.

wastes were being discharged of into the “River Chenab through Chakbandi Drain, and the other from areas of the River Chenab upstream of the drain outlet. These two categories were compared to the fish from farmed environments that were supposed to be pollution free. Water analysis showed significant changes in the water quality of the River Chenab downstream from the drain outlet. Fish from the highly polluted sites showed less fatty acids and more saturated fatty acids in their meat compared to fish from less polluted areas”. *C*. *mrigala* collected from polluted river sites and upstream sites before the entrance of Chakbndi Main Drain showed non-

significant differences in saturated and unsaturated fatty acids, signify higher sensitiveness for contamination due to its feeding niche. Polyunsaturated, mono-unsaturated and saturated fatty acids from highly polluted areas were 3.6±0.02, 1.2±0.02 and 15.2±0.88 g/100 g of dry meat respectively in *C. mrigala*. Polyunsaturated, mono-unsaturated and saturated fatty acids from upstream (before the Chakbandi Drain outlet) were recorded as 6.5±0.02, 0.2±0.02 and 13.7±0.76 g/100 g respectively, in *C. mrigala*. Polyunsaturated, mono-unsaturated and saturated fatty acids in fish from the controlled environment were determined as 8.3±0.18, 4.1±0.04 and 7.6±0.58 g/100 g respectively in *C. mrigala* (Table 4).

Fish from highly contaminated sites, also exhibited a less number of fatty acids in total "and more saturated fatty acids in their meat compared to fish from less polluted areas. C8:0, C12:0, C16:1 (n-7), C16:1 (n-9), C20:1 (n-9), C18:2 (n-6), C18:4 (n-3), C22:4 (n-6) fatty acids were all missing in fish from the highly polluted habitat where significant DNA damage was reported". The loss of these beneficial fatty acids may not be easily compensated by humans when the majority of the diet comprises of fish. Seven and eight fatty acids of the omega series were reported in the flesh of fish from high and low pollution intensity habitats, respectively, almost half of that reported from non-polluted environments. This study suggests, therefore, that the loss of essential unsaturated fatty acids is due to the habitat effect and this loss can only be reversed by using fish from a healthy environment or by improving the habitat as in the farmed fish unsaturated fatty acids showed dominance in all the meat samples (Swapna et al., 2010). The comparison between the above-mentioned sampling sites provides a clear domino effect indicating that this atmosphere has grown into a highly poisonous habitat and is probably not appropriate for the growth of fish capable of producing high quality meat

The findings of this study corroborate the results of Van Der Oost et al. (2003) and Mahboob et al. (2014) on the use of fish biomarkers as indices of the effect of pollution. *C. mrigala* showed a very restricted population in the study area and was observed to be near extinction as the habitat of the fish is not suitable for the growth of this species. The high fat content of fish (13.7±0.76 and 15.2±0.88 g/100 g of dry fish meat as in this case), "although help in the buoyancy as the density of the fat is lower than water, but it deters the movement of the fish even in lower levels of the pollution. Fish having high saturates accompanied by contaminants in the body requires more energy to perform daily activities, especially during locomotion in struggle of food (Kandemir and Polat, 2007)".

In this study, 97.40% people (living near the river only) identified and rejected the fish from unhealthy habitats due to its appearance and aroma. People now rely on farmed fish even though these have less polyunsaturated fatty acids (Hussain et al., 2011). Most of the current literature concerning the combined effects of heavy metals on fish mainly comes from histopathological and physiological studies, while information about potential genotoxic effects on this vertebrate group is still scarce (Galindo et al., 2010). In spite of strenuous efforts, we could not obtain any comprehensive information in the literature for the comparison of these findings related to DNA damage and fatty acid profile; this work's attempt to survey DNA damage and fatty acid profile together may, therefore, be considered as a baseline for future studies.

In respect to comet assay data specifically, our results for *C. mrigala* agree with those of Pavlica et al. (2011) in the context of environmental biomonitoring of genotoxicity in fish. Burger and Gochfeld (2005) also reported that higher level of heavy metals in the water of river Chenab not only accumulate in the fish body but also moved in the route of metabolizim, thus reduce the quality of the fish meat. Pietripiana et al. (2002), meanwhile, reported elevated levels of micronucleus induction in erythrocytes of fish collected from unhealthy habitats, but none of these studies related these findings to the fatty acid profile of the fish. It is possible that this combination of effects is among the main reason leading to the extinction of this fish species in polluted sites of the River Chenab: fish from non-polluted sites clearly depict higher concentration of omega-3 polyunsaturated fatty acids and acceptable concentrations of the saturated fatty acids.

4 CONCLUSION

River Chenab was found to be profoundly polluted by sewage and industrial waste disposal. *Cirrhinus mrigala* from this polluted area not only showed significant DNA damage in the body but also revealed devastation of essential fatty acids even in the habitats of lower intensity pollution. Fishes are preferred for their white meat with unsaturated fatty acids and the

degradation of habitat by pollution not only reduces fish population but also deteriorate meat quality. From the present findings it is concluded we have to restore our pollution free freshwater bodies for healthy foodstuff with required nutrients.

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