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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