Chemical composition and variation in fatty acids profile of different muscle zones (dorsal, ventral, ventricha and tail) of wild major carp *Labeo rohita*

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Abstract

Keeping in view the industrial and domestic sewage containing untreated water direct discharge into River Chenab via Chakbandi main drain (CMD), the current research project was planned with the objectives to determine the effect of freshwater pollution on physicochemical parameters, heavy metal determination in the River Chenab water and its effects on fatty acids profile in four muscle zones (dorsal, ventral, ventricha and tail) of wild major carp (*Labeo rohita*). Fish species were collected from two different sites of River Chenab one as less polluted site (LPS-upstream to CMD) and other as highly polluted site (HPS-downstream to CMD) while control samples were collected from Fish Hatchery, Satiana Road, Faisalabad. Fatty acid (FAs) analysis was performed by Gas Chromatography using Flame Ionization detector. A total of 24 FAs were recorded in this Fatty acid profile/analysis. Out of 24 FAs; there were 7 saturated fatty acids (SFA), 6 monounsaturated fatty acids (MUFA) and 11 polyunsaturated fatty acids (PUFA) in fish muscle. The SFA contents were found at a highest (77.772±0.472%) in dry meat in ventral muscle zone of *Labeo rohita* harvested from a highly polluted site (downstream) of river Chenab. In contrast, SFA contents were found at a lowest ((26.026±0.033%)) in the dorsal muscle zone of *Labeo rohita* harvested from commercial fish farmed closely followed by tail muscle zone (26.917±0.030%)and clear difference from upstream and downstream wild fish. The maximum concentration of MUFA contents were found in ventral muscle zone (21.560±0.032%) of *Labeo rohia* collected from commercial fish farmed as compared to upstream and downstream area of River Chenab. While in the same case of PUFA contents were found at highest (42.140±0.016%) in the ventral muscle zone of Rohu harvested from commercial fish farmed. In contrast the concentration of PUFA profile were found lowest in all muscle zones of wild fish collected from LPS and HPS of River

CenabThere was a significant (P<0.05) decrease in SFA composition of fish samples downstream of the river than farmed and upstream wild fish. . The fatty acids C8:0, C12:0, C20:0, C16:1(n-7), C16:1(n-9), C18:1(n-9), C20:1(n-9),C18:2(n-6), C22:4(n-6), C20:5(n-3) were not detected in all muscle zones of wild major carp *Labeo rohita* collected from HPS of River Chenab.

Keywords:

Labeo rohita, Water Pollution, Fatty Acids Profile, Saturated fatty acids, Polyunsaturated fatty acids, Monounsaturated fatty acids, Muscle zones

Introduction

The population of people will grow every day and is expected to reach 9 billion by the year 2050. Due to the fact that fish is the most affordable source of protein, it follows that as the population grows, there's also the need for food. Global fish output in 2018 was estimated to be 179 million tonnes, with aquaculture accounting for 46% of the overall production, according to the Food and Agriculture Organisation of the United Nations (FAO, 2020).

In humans, these fatty acids protect against arteriosclerosis, neurological illnesses, cardiovascular disease, and stroke (Turchini et al. 2010; Golden et al. 2021). (Safiin et al. 2021) report that palm oil has been utilised to substitute fish oil for diet in a number of farmed fish species. In addition to its delicious flavour, fish is in high demand because of its high-quality meat (Njinkoue et al. 2016). The species, diet, age, sex, environment, and season all affect its makeup. Due to low-fat fish and products made by fish are exclusively nourishing and valuable components of ɷ3 PUFAs, made in high proteins, long muscle filaments, liposoluble vitamins, essential minerals and all critical amino acids that are used for valuable food sources (Ali et al., 2019). The composition of fatty acids in fish and fish products is increasing in importance due to their dietary and several health benefits (Mahadevan et al., 2019). However, arachidonic acid (ARA) and linoleic acid (LA) are held by ɷ6 PUFAs, which are essential for maintaining human health (Robert et al., 2014). The Indian major Carps are strengths of freshwater aquaculture because these are the most preferred farmed fishes due to their fast growth and high acceptance by consumers due to having good taste and meat quality (Saini et al., 2014).

Indian major carp (IMC) species are leading the way in freshwater aquaculture in India. The highest growth rate, flavour, and public choice make IMCs, Catla, rohu and Mori, the most significant and main cultivable fish species in India. According to FAO 2022, rohu is among the top ten aquaculture species cultivated globally. It accounts for over 35% of India's total production of cultivated fish. In a year, it can reach a weight of 800–1000 g. Farmers in India have switched from three-species to two-species polyculture systems using rohu and catla because of the increased market value of rohu (FAO 2018). According to Rasal and Sundaray (2020), Rohu is a significant aquaculture species in India, Bangladesh, Pakistan, and Myanmar (Burma). This species was easily domesticated, had a high fecundity rate (2 lakh eggs/kg), and was externally fertilised (Rasal and Sundaray 2020). The growing market demand for rohu necessitates intensifying its culture, which calls for inexpensive, nutritionally balanced aquaculture feeds. According to Hussain et al. (2016), aquatic pollution may significantly decrease the quality of fish meat and cause the death of several water populations. Water body contamination is currently a global environmental issue Khan et al. (2016). Numerous toxic synthetic compounds, heavy metals, and other pollutants can be found in industrial effluents Zaqoot et al. (2017).

Wetlands have a serious pollution problem that could endanger the health and welfare of the entire community. Large amounts of liquid, gaseous, and solid waste are produced when large communities congregate in cities. Additionally, industries generate enormous amounts of garbage of various kinds. The process of treating industrial wastes has not kept up with the rise of industry, no matter how intense it has become. The harmful materials found in these wastes have the potential to damage aquatic life, upsetting entire ecosystems, and endanger human health either directly or indirectly Pappa et al. (2016). In addition to harmful metals that harm fish when they are released into water systems, industrial wastes typically contain high concentrations of dissolved and suspended particles, heavy metals, organic and inorganic compounds, high BOD and COD, oils, and grease. 50% of human waste is dumped into waterways, and most wetlands have been used for industrial purposes because of their inherent ability to absorb heavy metals and other pollutants. (Joyce 2012; Anawar and Chowdhury 2020).

This research was done to determine the pollution impacts on the water quality of the River Chenab and the fatty acid profile of different muscle zones of *Labeo rohita* collected from the River Chenab and farmed fish (control) due to the significance of these valuable species and the water of the Chenab River. Our study focused mostly on assessing the susceptibility of various muscle zones to pollutants.

Material and methods

Faisalabad is 3rd largest city of Pakistan and it contains 45% industries of country. Large quantities of toxic wastes from industries are disposed into two main rivers of Pakistan; River Chenab and River Ravi. This research was planned to study the effect of industrial and domestic wastewater on water quality of river Chenab and river fish. Faisalabad city is known as "Manchester" of Pakistan because of textile rich industries, mills and factories that releasing a huge quantity of toxic waste into River Chenab by Chakbandi Main Drain (CMD) at entrance of Thatta Muhammad Shah (Ahmad Wala) at latitude 31.570° and longitude 72.534° (Fig.1). River Chenab contains variety of aquatic fauna especially fishes as fishes are good source of protein for humans but contaminated water discharge in river Chenab continuously polluting fish. Domestic wastes and industrial effluents from CMD has drastically reduced fish population and even that is also inappropriate for irrigation use. This study predicts the potential for variation in muscle composition of fish meat in different muscle zones in response to freshwater pollution. Farmed fish was collected from Fish Seed Hatchery and designated as a control. Following the collection of all fish samples, the stomachs of the samples were cleared using tap water. The fish's dorsal, ventral, ventrecha, and tail muscule zones were then visible once the skin was peeled off. A polyethylene bag containing four distinct muscle zones was used to examine the fatty acid composition (Fig. 2).

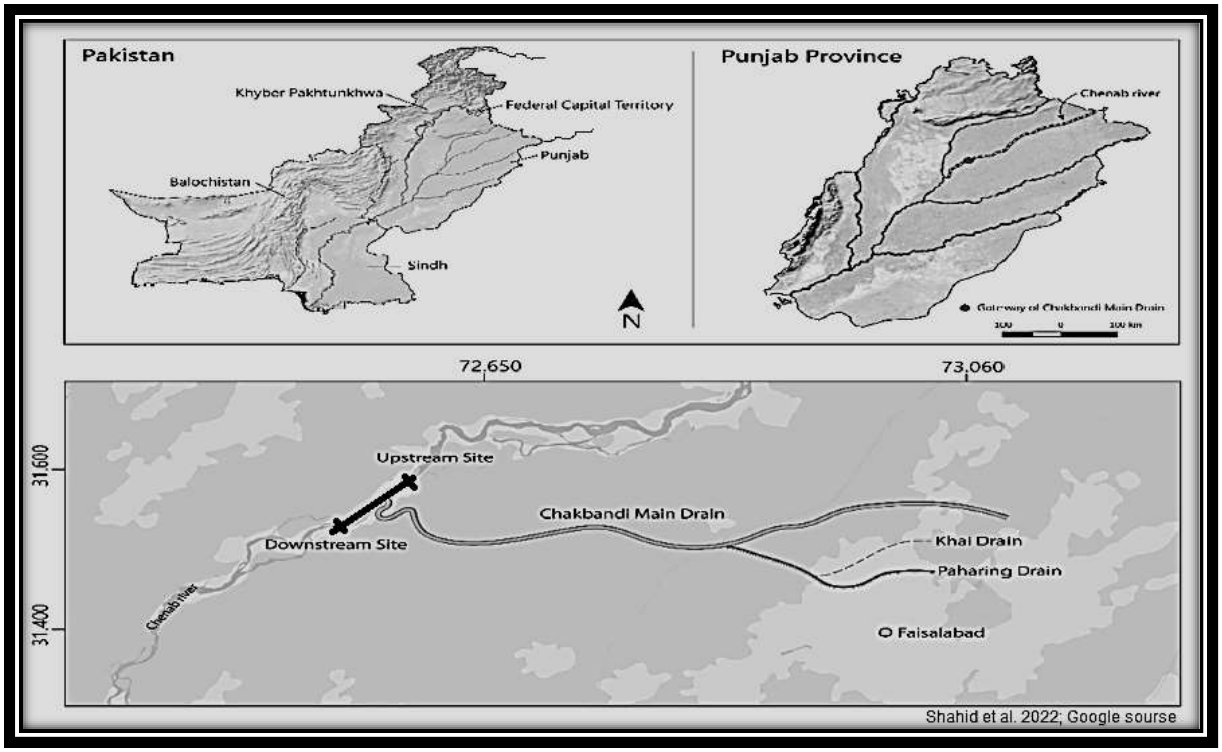

FIGURE 1. Site map of study area showing downstream and upstream study areas of River Chenab.

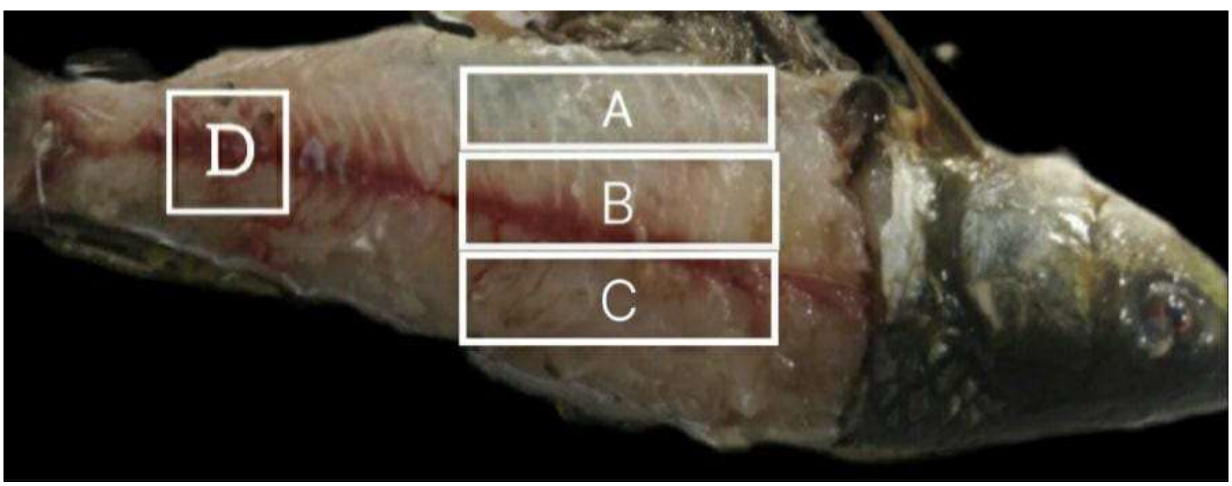

FIGURE 2. The study identifies the muscle zones of *Labeo rohita*, including the Ventral Zone (A), Ventricha Zone (B), Dorsal Zone (C), and Tail Zone (D).

Water analysis

Water samples were taken from the surface because *Labeo rohita* is a column feeder to estimate the pollution level in which harvested fish are inhabited. According to the Environmental Protection Agency of Pakistan, these water samples were examined for water quality and heavy metals. According to the (EPA 2015), these water samples were analysed for various metal concentrations and water quality indicators. Before being analysed, the water samples were kept cold at 4°C in a refrigerator after being preserved in 5 millilitres of 55% HNO3/L of water to prevent contamination. Every water quality parameter was established using the guidelines provided by Boyd 1981. Heavy metals named tin (Sb), chromium (Cr), lead (Pb), zinc (Zn), manganese (Mn), copper (Cu), cadmium (Cd), and mercury (Hg) were analyzed by Hitachi polarized Zeeman Atomic Absorption Spectrophotometer AAS, 2000 series.

Fatty Acid Analysis

The fatty acids profile in the muscle zones of all fish was determined by the Flame Ionization detector and technique of GC-MASS in the following phases. The fish samples were cleaned, peeled and extracted oil using n-hexane (MDA_CHEM-107023) and chloroform 50:50 (V/V) (Shahid, 1987). Oils were detected for FA contents using Gas Chromatography (Shimadzu-A 14, Japan) with a flame ionization detector (FID) and attached to an integrator. Fish samples were inserted 3 µL through carrier gases H_2, He and air 40, 40 and 500 mL1- minute correspondingly, injection port200°C, column 190°C, and identification port temperature 210°C. Every fish sample was studied three times, and that means were considered. **(**Hedayatifard and Jamali (2008). Fatty acids were identified by contrasting the retention periods of FAME (Supelco, Catalogue No. 18919) with the standard 37-component FAME mixture. The GC analyses were performed in replicates, and the results were represented as a percentage of the GC area as means values ^ standard deviations (SD).

Statistical analysis

ANOVA was performed on the two factors, and Tukey's test was used to compare the means at a significance level of 5%. The statistical analysis was performed out entirely using the SPSS 22 software.

Results

The parameters of water quality and the concentrations of heavy metals in the surface water of the River Chenab and commercial fish farms are shown in Table 1. Data was collected from a commercial fish farm at the Satiana Road Fish Hatchery, as well as two different site along the River Chenab: one upstream and other downstream that was heavily contaminated. The results of the experiment revealed that the concentration of heavy metals and other water quality parameters in the water taken from the River Chenab exceeded the WHO's (1992) permissible limit. The highest heavy metal concentrations were recorded in a water sample collected from a highly polluted site of River Chenab. The heavy metals were recorded in the following order: Mn (2.071±0.025) > Pb (2.031±0.03) > Cu (1.631±0.028) > Hg (0.829±0.015) > Cr (0.469±0.0007) > Sn (0.429±0.010) > Zn (0.361±0.013) > Cd (0.119±0.008) mg/L were recorded in highly polluted area of River Chenab in descending order compared with less polluted area and farm water with almost normal values.

The data shown for variation in fatty acids profile of total lipids were limited for different muscle zones of fish species *Labeo rohita* from two habitat. One from highly polluted site of River Chenab, where industrial and domestic waste water were being discharged into river through chakbandi drain and other from upstream area as less polluted site of River Chenab before enter of chakbandi drain into water. These two categories were being compared to same fish species from commercial fish farm supposed to pollution free environment. A total of 24 fatty acids were scored in this Fatty acid profile/analysis. There were 7 saturated fatty acids (SFA) (C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C20:0) 6 monounsaturated fatty acids (MUFA) (C16:1(n-7), C16:1(n-9), C18:1(n-7), C18:1(n-9), C20:1(n-9), C22:1(n-9) and 11 polyunsaturated fatty acids (PUFA, C18:2(n-6), C18:3(n-3), C18:4(n-3), C20:2(n-6), C20:4(n-6), C20:5(n-6), C20:5(n-3), C22:4(n-6), C22:5(n-3), C22:5(n-6), C22:6(n-3) were recorded in wild major carps among four muscle zones (Dorsal, Ventral, Ventricha and Tail) collected from LPS, HPS and farmed environment. The individual details of these all fatty acids in *Labeo rohita* collected from three sites were described below:

Overall, maximum fatty acid contents/concentration were found in fish collected from a less polluted area (upstream) followed by farmed and highly polluted site fish, respectively. A significant ($p<0.05$) difference in fatty acid profile in different muscle zones (dorsal, ventral, ventrecha and tail) of *Labeo rohita* were recorded among the fish samples harvested from different habitats. The contents of SFAs were found highest (77.772±0.472%) in dorsal muscle zone of *Labeo rohita* harvested from a highly polluted site (downstream) of the River Chenab and ventricha muscle zone (75.798±0.032%) closely followed by tail muscle zone (75.082±0.000%) of *Labeo rohita*. The minimum concentration of SFAs (59.538±0.016%) were recorded in the ventral muscle zone of fish samples collected from the selected upstream location of the River Chenab. Contents of SFAs were observed maximum in dorsal muscle zone (64.342±0.010%) closely followed by tail muscle zone (63.788±0.016%) of upstream wild fish harvested from River Chenab. The contents of MUFAs were found highest (5.280±0.063%) in dorsal muscle zone of *Labeo rohita* harvested from a less polluted site (upstream) of the River Chenab, closely followed by highly polluted site (downstream) (5.126±0.014%). The concentration of MUAs were recorded in all muscle zones of fish samples approximately same harvested from commerical fish farmed. Contents of PUFAs were observed maximum in ventral muscle zone of downstream wild fish (21.245±0.006%) harvested from highly polluted site of the River Chenab. The contents of PUFAs were found highest (42.140±0.016%) in ventral muscle zone of *Labeo rohita* collected from a commercial fish farmed but ventricha and tail muscle zone (41.720±0.016%, 41.810±0.047%) closely followed by each other.

The concentration of SFA C12:0 was found in ascending order among four muscle zones venctricha, tail, dorsal and ventral (2.730±0.016% < 2.750±0.016% < 2.960±0.016% < 3.030±0.016%) of *Labeo rohita* collected from commercial fish farm (Table 2). The concentration of SFA C16:0 (Palmitic acid) was found in decreasing order among ventral, ventricha, tail and dorsal (55.230±0.016% > 54.960±0.016% > 53.760±0.016% > 51.440±0.016%) muscle zones of *Labeo rohita* collected from HPS of River Chenab (Table 2). The highest concentration in farmed fish was found in muscle zone ventricha (0.310±0.142%) as compared to other zones dorsal, ventral and tail (0.170±0.016%, 0.190±0.016%, 0.250±0.016%) respectively (Table 2). Farmed *Labeo rohita* was shown in increasing sequence the concentration of C18:1n7 in dorsal (3.070±0.016%) < ventral (3.230±0.016%) ventricha (3.360±0.016%) < tail (3.430±0.016%) muscle zones respectively (Table 3). Overall maximum concentration of MUFA C22:1n9 (Erucic acid) were noted in following sequence in ventricha muscle zones (5.770±0.016 > 0.540±0.016 > 0.350±0.016%) compared to ventral (5.230±0.016 > 0.450±0.016 > 0.430±0.016%), tail (4.950±0.016 > 0.350±0.016 > 0.340±0.016%) and dorsal (4.750±0.016 > 0.360±0.016 > 0.260±0.016%) muscle zones of *Labeo rohita* collected from three habitats (HPS of River Chenab > LPS of River Chenab > Farmed fish) respectively (Table 3). The concentration of C18:2n6 were non-significant ($p>0.05$) among ventral and ventricha but the concentration of C18:2n6 was highly significant ($p<0.01$) among dorsal and tail muscle zone of *Labeo rohita* harvested from LPS of River Chenab (Table 3). While the highest concentration of PUFA C18:2n6 in farmed *Labeo rohita* was recorded in following sequence in ventral (2.850±0.016%) > ventricha (2.760±0.016%) > tail (2.650±0.016%) > dorsal (2.560±0.016%) muscle zones (Table 4). The highest concentration of PUFA C20:5n6 was recorded in following sequence in ventral (3.024±0.002%), ventricha (2.985±0.002%) tail (2.767±0.002%) and dorsal (2.565±0.002%) muscle zones of *Labeo rohita* collected from HPS of River Chenab (Table 4). The concentration of C22:6n3 (Docosahexaenoic acid) was non-significant ($p>0.05$) in all muscle zones of *Labeo rohita* collected from HPS of River Chenab (Table 4).

Table: 1 Comparison of means (Mean± SE) for water quality parameters.

Water Parameters	Highly Polluted Area	Less Polluted Area	Farmed	Desirable limits	Permissible limits
PH	11.71±0.071A	8.49±0.86B	7.61±0.11C	6.5-8.5	**, 6-10*
BOD (mg/L)	76.38±0.91A	44.65±0.74B	35.61±0.31C	30mg/L	**, 80 mg/L*
COD (mg/L)	188.20±1.71A	69.60±1.13B	60.20±0.69C	250mg/L	**, 150 mg/L*
TDS (mg/L)	2379.20±39.14A	1297.00±17.11B	324.39±5.31C	500mg/L	2000mg/L, 3500 mg/L*
TSS (mg/L)	309.00±6.01A	202.00±4.21B	166.60±2.34C	100mg/L	**, 150 mg/L*
Salinity (mg/L)	1905.00±21.41A	407.00±7.21B	206.21±3.19C	-	<100mg/L
Conductivity (mS/m)	3.222±0.031A	1.349±0.021B	0.336±0.010C	650µS/cm	1055µS/cm
Phenol (mg/L)	2.341±0.041A	0.850±0.011B	0.178±0.015C	0.001mg/L	0.002mg/L, 0.1 mg/L*
Sulphate (mg/L)	424.40±8.01A	327.00±7.17B	82.00±1.86C	0.001mg/L	0.002mg/L, 600 mg/L*
		Heavy metals			
Tin (Sn)	0.429±0.013A	0.002±0.000B	0.001±0.000B	0.01mg/L	**
Chromium(Cr)	0.469±0.003A	0.254±0.005B	0.036±0.002C	0.05mg/L	**, 1.0 mg/L*
Lead (Pb)	2.031±0.031A	0.232±0.013B	0.070±0.004C	0.05mg/L	**, 0.5 mg/L*
Zinc (Zn)	0.361±0.013A	0.150±0.007B	0.031±0.001C	5mg/L	15mg/L, 5.0 mg/L*
Maganese (Mn)	2.070±0.021A	1.752±0.011B	0.250±0.004C	0.1mg/L	0.3mg/L
Cupper (Cu)	1.631±0.022A	0.862±0.013B	0.051±0.004C	0.05mg/L	1.5mg/L, 1.0 mg/L*
Cadmium (Cd)	0.119±0.005A	0.070±0.006B	0.003±0.005C	0.01mg/L	**, 0.1 mg/L*
Mercury (Hg)	0.831±0.011A	0.007±0.003B	0.001±0.001B	0.001mg/L	**, 0.01 mg/L*

Means sharing similar letters for water quality parameters of different areas in a row are non-significant (P>0.05). BOD: Biological oxygen demand; COD: Chemical oxygen demand; TDS: Total dissolved solids; TSS: Total suspended solids; *EPA Pak ** No relaxation WHO

Table: 2 Effect of different quality of water on (% total saturated fatty acid) profile (Mean±S.E) in different muscle zones of *Labeo rohita*

Saturated Fatty acids	Area	Muscle Zones			
		Dorsal	Ventral	Ventricha	Tail
C8:0	Less polluted	0.007±0.002b	0.003±0.002bc	0.003±0.002bc	0.005±0.002bc
	Highly polluted	0.000±0.000c	0.000±0.000c	0.000±0.000c	0.000±0.000c
	Farmed	0.006±0.002bc	0.130±0.016a	0.003±0.002bc	0.007±0.002b
C10:0	Less polluted	0.330±0.016k	0.350±0.016j	0.380±0.016i	0.270±0.016l
	Highly polluted	7.225±0.002d	7.745±0.002b	7.433±0.002c	7.775±0.002a
	Farmed	2.730±0.016h	2.750±0.016g	2.930±0.016f	2.960±0.016e
C12:0	Less polluted	0.003±0.002e	0.005±0.002e	0.005±0.002e	0.003±0.002e
	Highly polluted	0.000±0.000e	0.000±0.000e	0.000±0.000e	0.000±0.000e
	Farmed	2.960±0.016b	3.030±0.016a	2.730±0.016d	2.750±0.016c
C14:0	Less polluted	1.460±0.016f	1.350±0.016f	1.430±0.016f	1.380±0.016f
	Highly polluted	2.467±0.002e	3.767±0.448bc	2.565±0.002e	2.877±0.002d
	Farmed	3.670±0.016c	3.870±0.016ab	3.960±0.016a	4.030±0.016a
C16:0	Less polluted	39.650±0.016f	36.930±0.016h	37.130±0.016g	40.030±0.016e
	Highly polluted	51.440±0.016d	55.230±0.016a	54.960±0.016b	53.760±0.016c
	Farmed	12.760±0.016l	13.760±0.016j	13.850±0.016i	12.960±0.016k
C18:0	Less polluted	22.862±0.019a	20.850±0.016d	21.760±0.016c	22.030±0.016b
	Highly polluted	10.160±0.016h	11.030±0.016e	10.840±0.016f	10.670±0.016g
	Farmed	3.730±0.016k	3.960±0.016j	4.030±0.016i	3.960±0.016j
C20:0	Less polluted	0.030±0.016de	0.050±0.016de	0.040±0.016de	0.070±0.016d
	Highly polluted	0.000±0.000e	0.000±0.000e	0.000±0.000e	0.000±0.000e
	Farmed	0.170±0.016c	0.190±0.016c	0.310±0.142a	0.250±0.016b
∑SFA	**Less polluted**	**64.342±0.010e**	**59.538±0.016h**	**60.748±0.016g**	**63.788±0.016f**

Saturated Fatty acids	Area	Muscle Zones			
		Dorsal	Ventral	Ventricha	Tail
	Highly polluted	**71.292±0.000d**	**77.772±0.472a**	**75.798±0.032b**	**75.082±0.000c**
	Farmed	**26.026±0.033k**	**27.690±0.016i**	**27.813±0.211i**	**26.917±0.030j**

Means sharing similar letters in a row or in a column within fatty acids are statistically non-significant (P>0.05)

Table: 3 Effect of different quality of water on (% total Mono- unsaturated fatty acid) profile (Mean ±S.E) in different muscle zones of *Labeo rohita*

Mono-unsaturated Fatty acids	Area	Muscle Zones			
		Dorsal	Ventral	Ventricha	Tail
C16:1(n-7)	Less polluted	0.000±0.000c	0.000±0.000c	0.000±0.000c	0.000±0.000c
	Highly polluted	0.000±0.000c	0.000±0.000c	0.000±0.000c	0.000±0.000c
	Farmed	0.040±0.016b	0.062±0.024a	0.040±0.027b	0.044±0.021b
C16:1(n-9)	Less polluted	0.740±0.016d	0.540±0.016g	0.630±0.016f	0.670±0.016e
	Highly polluted	0.000±0.000h	0.000±0.000h	0.000±0.000h	0.000±0.000h
	Farmed	1.960±0.016c	2.130±0.016a	2.070±0.016b	2.060±0.016b
C18:1(n-7)	Less polluted	0.050±0.016i	0.070±0.016h	0.030±0.016j	0.040±0.016ij
	Highly polluted	0.376±0.002g	0.396±0.002f	0.416±0.002e	0.423±0.002e
	Farmed	3.070±0.016d	3.230±0.016c	3.360±0.016b	3.430±0.016a
C18:1(n-9)	Less polluted	4.130±0.016e	3.850±0.016h	3.930±0.016g	4.070±0.016f
	Highly polluted	0.000±0.000i	0.000±0.000i	0.000±0.000i	0.000±0.000i
	Farmed	12.140±0.016a	11.870±0.016b	11.760±0.016c	10.960±0.016d
C20:1(n-9)	Less polluted	0.000±0.000e	0.000±0.000e	0.000±0.000e	0.000±0.000e
	Highly polluted	0.000±0.000e	0.000±0.000e	0.000±0.000e	0.000±0.000e
	Farmed	3.360±0.016d	3.830±0.016a	3.760±0.016b	3.540±0.016c
C22:1(n-9)	Less polluted	0.360±0.016g	0.430±0.016f	0.350±0.016g	0.340±0.016g

	Highly polluted	4.750±0.016d	5.230±0.016b	5.770±0.016a	4.950±0.016c
	Farmed	0.260±0.016h	0.450±0.016f	0.540±0.016e	0.350±0.016g
∑MUFA	**Less polluted**	**5.280±0.063g**	**4.890±0.032j**	**4.940±0.032i**	**5.120±0.032h**
	Highly polluted	**5.126±0.014h**	**5.626±0.014e**	**6.186±0.014d**	**5.373±0.017f**
	Farmed	**20.860±0.063b**	**21.560±0.032a**	**21.530±0.063a**	**20.370±0.032c**

Means sharing similar letters in a row or in a column within fatty acids are statistically non-significant (P>0.05)

Table 4: Effect of different quality of water on (% total Poly- unsaturated fatty acid) profile (Mean ±S.E) in different muscle zones of *Labeo rohita*

Poly-unsaturated Fatty acids	Area	Muscle Zones			
		Dorsal	Ventral	Ventricha	Tail
C18:2(n-6)	Less polluted	0.270±0.016g	0.370±0.016e	0.360±0.016e	0.330±0.016f
	Highly polluted	0.000±0.000h	0.000±0.000h	0.000±0.000h	0.000±0.000h
	Farmed	2.560±0.016d	2.850±0.016a	2.760±0.016b	2.650±0.016c
C18:3(n-3)	Less polluted	0.005±0.002i	0.003±0.002i	0.003±0.002i	0.005±0.002i
	Highly polluted	2.650±0.016g	2.760±0.016f	2.470±0.016h	2.850±0.016e
	Farmed	3.230±0.016a	3.030±0.016d	3.070±0.016c	3.130±0.016b
C18:4(n-3)	Less polluted	2.430±0.016a	2.340±0.016b	2.410±0.016a	2.340±0.016b
	Highly polluted	1.760±0.016g	1.960±0.016d	2.030±0.016c	1.870±0.016e
	Farmed	1.750±0.016g	1.950±0.016d	1.830±0.016f	1.870±0.016e
C20:2(n-6)	Less polluted	0.050±0.016g	0.040±0.016gh	0.030±0.016h	0.040±0.016gh
	Highly polluted	0.687±0.002f	0.794±0.002d	0.746±0.002e	0.817±0.002c
	Farmed	0.690±0.016f	0.950±0.016a	0.730±0.016e	0.840±0.016b
C20:4(n-6)	Less polluted	0.860±0.016i	0.830±0.016j	0.760±0.016k	0.770±0.016k
	Highly polluted	8.347±0.002g	9.023±0.002e	8.983±0.002f	8.243±0.002h
	Farmed	10.770±0.016d	10.960±0.016c	11.030±0.016b	11.130±0.016a
C20:5(n-6)	Less polluted	8.030±0.016c	7.930±0.016d	8.450±0.016a	8.130±0.016b
	Highly polluted	2.565±0.002l	3.024±0.002i	2.985±0.002j	2.767±0.002k

	Farmed	3.170±0.016h	3.360±0.016f	3.250±0.016g	3.540±0.016e
C20:5(n-3)	Less polluted	2.760±0.016h	2.850±0.016g	3.030±0.016f	3.140±0.016e
	Highly polluted	0.000±0.000i	0.000±0.000i	0.000±0.000i	0.000±0.000i
	Farmed	3.950±0.016d	4.130±0.016a	4.050±0.016c	4.070±0.016b
C22:4(n-6)	Less polluted	0.000±0.000d	0.000±0.000d	0.000±0.000d	0.000±0.000d
	Highly polluted	0.000±0.000d	0.000±0.000d	0.000±0.000d	0.000±0.000d
	Farmed	0.330±0.016c	0.460±0.016a	0.450±0.016a	0.370±0.016b
C22:5(n-3)	Less polluted	1.770±0.016j	1.840±0.016i	1.760±0.016j	1.830±0.016i
	Highly polluted	3.213±0.002h	3.423±0.002e	3.347±0.002f	3.245±0.002g
	Farmed	3.550±0.016d	3.840±0.016a	3.760±0.016b	3.630±0.016c
C22:5(n-6)	Less polluted	4.850±0.016b	5.030±0.016a	4.780±0.016c	4.840±0.016b
	Highly polluted	0.156±0.002i	0.145±0.002i	0.125±0.002j	0.178±0.002h
	Farmed	3.630±0.016f	3.760±0.016e	3.840±0.016d	3.550±0.016g
C22:6(n-3)	Less polluted	3.560±0.016h	3.830±0.016f	3.740±0.016g	4.030±0.016e
	Highly polluted	0.106±0.002i	0.116±0.002i	0.107±0.002i	0.106±0.002i
	Farmed	6.540±0.016d	6.850±0.016c	6.950±0.016b	7.030±0.016a
PUFA	**Less polluted**	**24.585±0.017h**	**25.063±0.046g**	**25.323±0.017f**	**25.455±0.109e**
	Highly polluted	**19.484±0.035l**	**21.245±0.006i**	**20.793±0.003j**	**20.076±0.003k**
	Farmed	**40.170±0.079d**	**42.140±0.016a**	**41.720±0.016c**	**41.810±0.047b**

Means sharing similar letters in a row or in a column within fatty acids are statistically non-significant (P>0.05).

Discussion

Our results showed that all water quality parameters and concentration of heavy metals in water collected from river Chenab were greater than (WHO 1992). permissible limit. The highest water quality parameters and heavy metal concentrations values were recorded in a water sample collected from a highly polluted site on River Chenab. Our results are supported by Hussain et al. (2016) reported that River Chenab is highly polluted with heavy metals due to industrial runoff and domestic sewage discharge through the Chakbandi drain, in River Chenab was also above the permissible limits.Few studies have investigated the water quality and heavy metal pollution affecting the freshwater ecosystem in different areas of Pakistan (Bhowmik et al., 2015, Hussain et al., 2014). Our results showed that the highest heavy metal concentrations were recorded in a water sample collected from a highly polluted site of River Chenab. Different studies reported that in case of heavy metals, concentration of lead was found maximum at Satowali and exceeding the acceptable limits at all sampling stations Dogra et al. (2023). A relationship of heavy metal levels in various tissues of fish and DNA fragmentation. The gills are a metabolically active organ in fish and can accumulate more heavy metals than other organs Sultana et al. (2019). Our results are supported by Kumar et al. (2020) the Pb content of all the water bodies exceeded the limit for EPR, BIS and WHO guidelines for drinking water. The heavy-metal pollution index was also applied to the contents of different heavy metals to find which water body is more polluted, and from their results, it was found that Harike Wetland and Sutlej River are critically polluted with heavy metals. The Pb, Cu, Zn, and Cr content were high in the Harike wetland and Sutlej River which could be overcome by introducing some good phytoremediator aquatic plants Bai et al. (2011). Heavy metals catalyze reactions that generate reactive oxygen species, which may cause oxidative stress and damage to tissues and muscle proteins, and lipids. Our findings are in line with the results of other studies **(**Mahboob et al., 2011**;** Chaudhry and Jabeen, 2011**;** Hussain et al., 2016**).**

Our results showed that saturated fatty acids (SFAs) composition in the four muscle zone of *Labeo rohita* varied according to fish habitat change. The fish harvested from the downstream location of the river showed a significant ($P<0.05$) increase in SFAs contents compared to upstream wild and farmed fish. This finding are similar to Simat et al. (2020) the sum of the saturated fraction was the highest from July to September, with palmitic acid (C16:0) being predominant, followed by myristic (C14:0), and stearic fatty acid (C18:0). Palmitic acid reached levels of 2.19, 1.66, and 1.61 g/100 g of EP in July, August, and September, respectively. The lowest saturated fatty acids (SFA) were tricosylic acid (C23:0; 0.01 g/100 g) and arachidic acid (C20:0; 0.02 g/100 g). Our finding showed that the concentration of C18:2n6 were non-significant ($p>0.05$) among ventral and ventricha but the concentration of C18:2n6 was highly significant ($p<0.01$) among dorsal and tail muscle zone of *Labeo rohita* harvested from LPS of River Chenab. In fatty acid profile of pirarucu, C18:1 was the main fatty acid, followed by C16:0 and C18:0. Castro et al. (2007) observed a similar profile in three freshwater fishes. According to Food and Agriculture Organization of the United Nations (2009), generally, C18:1 is the main fatty acid present in products of animal origin so as vegetal origin. Other different studies reported that the major SFA reported in sardines were palmitic acid (C16:0), followed by myristic acid (C14:0) and stearic acid (C18:0). The dominant MUFA and PUFA were identified as palmitoleic acid (C16:1 *n*-7), oleic acid (C18:1 *n*-9), EPA, and DHA. The seasonal changes in FA result from the feeding activity of sardines and the seasonal variations in plankton, since the FA profile from sardines corresponded to that of plankton (Zlatanos & Laskaridis, 2007, Bouderoua et al., 2011, Zotos & Vouzanidou, 2012).

Our current findings about the concentration of fatty acids in different muscle zones revealed that overall maximum fatty acids were found in the ventral muscle zone than in other muscle zones (dorsal, ventricha, and tail) from all fish samples collected in three diversified habitats. These findings were in line with Martins et al. (2017), who reported that protein and total lipids were found maximum in ventrecha muscle than in dorsal, ventral, and tail muscle in studied fish (pirarucu).

Conclusion

This study endorses the non-homogeneous composition of FAs contents in different muscle zones in *Labeo rohita*. Maximum FAs profile were found in the ventral muscle zone in all fish samples collected from diversified study areas showing that this muscle region is best for meat composition and more resistant to pollution than other muscle zones. The study recommends that upstream wild and farmed fish have good FAs profile and are best for consumption. However, it is pretty evident from our study that river fish have better nutritional composition due to a wide range of natural diets if it is free from contaminants or within a permissible limited set by WHO. In this study, we faced a scarcity of literature regarding distributional variation in FAs profile in different muscle zones of fish in response to surrounding water pollution to determine the muscle zone sensitivity to pollution. So, sustainability management requires no compromise for untreated industrial effluents and domestic sewage before discharge into the river. Our study would create public awareness about nutritional quality and variation of concentration of FAs in different muscle zones in response to pollution. Besides this, our study would be helpful for policymakers to enforce laws to mitigate pollution in rivers.

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Conflict of Interest

All the authors declare that they have no any conflict of interest.

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