Impact of Industrial Effluents on Amino acids Profile of different muscle zones of *Catla catla* inhabitants of River Chenab

Anees Hayat[1], Tayyaba Sultana[1], Salma Sultana[1], Bilal Hussain[1], Saddam Hussain[2]

[1] *Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad, Pakistan.*
[2] *Department of Zoology, Faculty of Basic Sciences, University of Agriculture, Dera Ismail Khan, Pakistan.*

*** Corresponding author:** Prof. Dr. Tayyaba Sultana

Abstract: The present study aimed to assess the impact of pollution on the amino acid profile of different muscle zones (Dorsal, Ventral, Ventricha, and Tail) of *Catla catla* (column feeder) by paper chromatography. Fish samples were collected from an area of non-polluted (upstream) sites and polluted sites (downstream) near Thatta Muhammad Shah, where the Chakbandi Main drain joins River Chenab. The essential amino acids contents were found highest (0.1349±0.00023g/g) in dry meat in the ventricha muscle zone of *Catla catla* harvested from a less polluted site (upstream) of river Chenab closely followed by farmed fish (0.0983±0.00057g/g). In contrast, essential amino acid contents were found at a minimum (0.0249±0.0004g/g) in the ventral muscle zone of *Catla catla* harvested from a highly polluted site (downstream) of the river Chenab clear difference from upstream wild and farmed fish. There was a significant ($P<0.05$) decrease in the amino acid composition of fish samples downstream of the river than farmed and upstream wild fish. Among essential amino acids, threonine, arginine, and non-essential amino acids, Proline was found missing in all muscle zones of fish from highly polluted river areas.

Keywords: amino acids profile, *Catla catla*, muscle zones, water pollution

Introduction:

The human population will increase daily, and it is estimated that it will increase to 9 billion in 2050. So obviously, the demand for food with an increase in population is also rising, including the consumption of fish because fish is the cheapest source of protein (FAO, 2020). Fish meat has high nutritional value as it contains essential amino acids and unsaturated fatty acids in maximum proportion (Mohanty et al., 2019) that are required from food and easily digestible (Lise et al. 2021). Consumption of fish decreases the effects on the respiratory system, immune system, and

brain functions due to a lack of protein in the body (Simat et al., 2020). High demand of fish is not only due to its good taste but due to having good meat quality (Njinkoue et al., 2016). This composition varies on the species, diet, age, sex, environment, and season (Ondo-Azi et al., 2013). The amino acids in fish proteins have a significant role in a different phenomenon that benefits consumers' health. Glutamic acid, aspartic acid, and glycine enhance the healing of wounds, and tyrosine, methionine, histidine, lysine, and tryptophan, efficiently remove radicals in oxidative reactions (Ryu et al., 2021).

The Indian major Carps are a mainstay of freshwater aquaculture because these are the most preferred farmed fishes due to their fast growth and high acceptance by consumers (Saini et al., 2014). However, it has been proved that consuming contaminated fish has health risks for humans due to water pollution. As a means of human activities, the rapid growth of industries is the primary cause of water pollution worldwide, especially in cities. Rivers act as sinks for industrial effluents (Kumar et al., 2020) and municipal wastes (Chinedu and Chukwuemeka, 2018; Prakash and Singh 2020). As a result, the water quality decreases (Gebre et al., 2016) by increasing levels of metals in water and disturbs different life stages of aquatic organisms (Merola et al., 2021). Ultimately it causes accumulation in water sediments (Zafarzadeh et al., 2018) that are highly persistent (Ali et al., 2018) and results in bio magnification through the food chain (Khan et al., 2016; Nascimento et al., 2017; Zaqoot et al., 2017). Accumulating toxic metals in fish muscle can lead to many disorders (Kumar et al., 2019; Ali et al., 2020). The consumption of fish reared in a toxic environment causes many diseases, e.g., anuria, nephritis, and extensive lesions (Kjellan et al., 2015; Proshad et al., 2018). Exposure of fish to Cu and Li caused over-expression of the free amino acids (Lane et al., 2019). Copper can bind with Sulphonyl, carboxyl, and imidazole groups of amino acids; therefore, it causes the inactivation of protein (Filimonova et al., 2016). As a result, contaminated river waters are inappropriate for consumption by humans, fisheries, and agricultural users (Proshad et al., 2020). The health risks related to consuming fish that accumulates heavy metals in their bodies reduce the nutritional benefits that may be obtained from fish (Ayanda et al., 2019). The carps are an essential source of aquatic food; especially major carps are commercially important, and these fish species are widely cultured in Pakistan. The major Carps include Thaila (*Catla catla*), Rohu (*Labeo rohit*a), and Morakhi (*Cirrhinus mrigala*) are good sources of protein from Pakistani rivers (Sheikh et al., 2017).

Owing to the importance of these valuable species and the significance of Chenab river water, this research work was performed to determine the pollution impacts on the water quality of River Chenab and the amino acids profile of different muscle zones of *Catla catla* collected from River Chenab and farmed fish (control). The primary emphasis of our research was to evaluate the pollution sensitivity of different muscle zones.

Materials and Methods

Catla catla was harvested from a highly polluted area, and the other was a less polluted area (upstream) of the river Chenab. The highly polluted site was selected from Thatta Muhammad Shah to Trimu Head in district Jhang, Pakistan. This selected area has high pollution due to the high load of industrial effluents and sewage discharged via the Chakbandi drain with a latitude of 31°34'13.5"N and a longitude of 72°32'03.4"E. A less polluted area was selected at Thatta Muhammad Shah at the point of Thalli of the river before the Chakbandi drain entered the river (Fig. 1). Farmed fish was collected from Fish Seed Hatchery and designated as a control. Fish samples were collected from three selected areas for estimation of amino acids profile in different muscle zones (Dorsal, Ventral, Ventricha, and Tail). After collecting all fish samples, all samples were cleaned with tap water to make the stomach empty. Then fish skin was peeled off, so muscle zones (Dorsal, Ventral, Ventricha, and Tail) were observed. Four muscle zones were separated and put in a polyethylene bag to analyze the amino acid profile (Fig. 2).

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

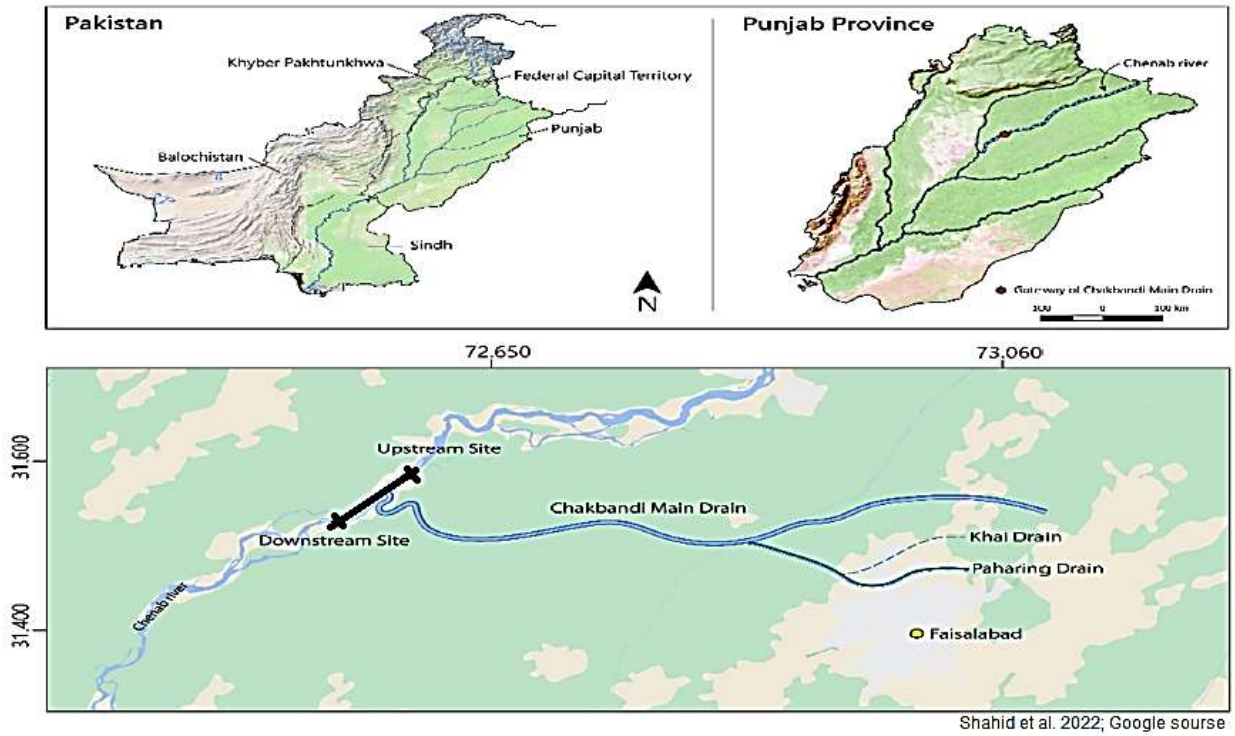
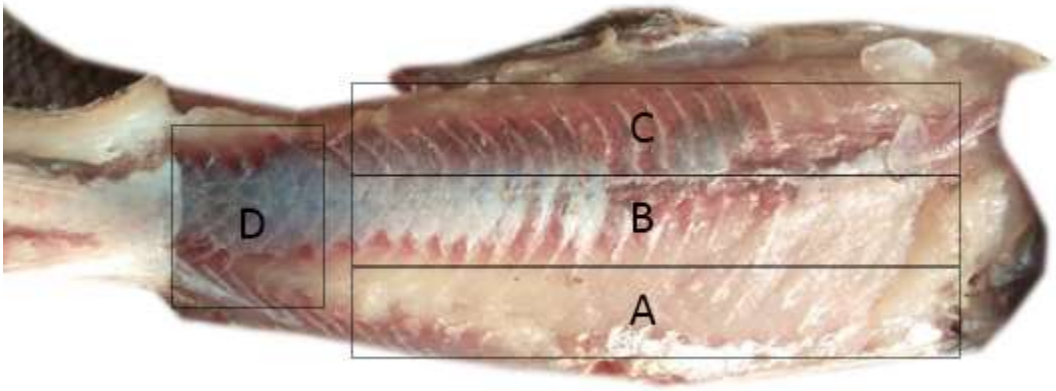


FIGURE 2. Marking of Muscle Zones of *Catla catla* for Ventral Zone (A) Ventricha Zone (B) Dorsal Zone (C) Tail Zone (D)



Water Analysis:

Water samples were collected from the exact location from where fish was harvested. Water samples were taken from the surface because *Catla catla* is a surface feeder to estimate the pollution level in which harvested fish was inhabited. These water samples were examined for

water quality parameters and concentration of different metals according to the (Environmental Protection Agency of Pakistan, 2015). The water samples were preserved in 5 ml of 55% HNO3/L of water to avoid contamination and then stored in the refrigerator at 4℃ before analyses. All quality parameters of water were determined according to defined 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.

Amino acid analysis

Paper chromatography determined the amino acid profile from all fish muscle zones (Fig. 2) (Hussain et al. 2016). This process was performed in four phases' hydrolysis, paper chromatography, paper electrophoresis, and Quantitative estimation. In hydrolysis, all weighed samples of dry ground meat were sealed in a glass tube ampoule with 6 M HCL and heated at 110℃ for 24 hours. In this step, peptide bonds between amino acids were broken. The ampoule tube broke then all solvent evaporated, leaving behind free amino acids. In the paper chromatography paper strip, Desaga NR 2045/ Whatman 1 was spotted with a sample by a capillary tube irrigated in n-butanol: acetic acids for 18-20 hours. The paper dried at 90℃. Then it was sprayed with 0.1 % ninhydrin ethanolic solution. After that, amino acids were identified by comparing Rf values of spots with standards of amino acids. After performing paper chromatography, performed paper electrophoresis to separate and distinguish those spots that are so closed and not separated by paper chromatography. These spots were irrigated in phosphate buffer (pH 7.0), and a 1.5 kV charge was applied for 1 hour. The paper was placed for 15 minutes at 70℃ and then sprayed with the 0.1% ninhydrin solution. The paper was dried for 10 minutes at 70℃. The appearance of purple spots was compared with the standard of amino acids for confirmation. So after completion of paper chromatography, paper electrophoresis was performed. In this step, color spots on the paper were cut and eluted in 3ml of methanol. After that, optical density was measured at 550nm. The quantitative result was recorded by comparing the curve to relevant Amino Acids standards (Grable et al., 1964).

Results

The water quality parameters and heavy metal concentrations in the surface water of the river Chenab and commercial fish farm are shown in Table 1. Data was collected from two different

sites of River Chenab, one highly polluted site (downstream) and a less polluted site (upstream), and a commercial fish farm at Satiana road fish hatchery. This result showed that these water quality parameters and concentration of heavy metals in water collected from river Chenab were greater than (WHO's 1992) permissible limit. The highest heavy metal concentrations were recorded in a water sample collected from a highly polluted site on River Chenab. The heavy metals were recorded in the following order: Mn (2.070±0.025) > Pb (2.032±0.03) > Cu (1.630±0.028) > Hg (0.830±0.015) > Cr (0.470±0.0007) > Sn (0.430±0.010) > Zn (0.360±0.013) > Cd (0.120±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.

Seventeen amino acids were present in different muscle zones of studied fish samples with different concentrations. Out of these, ten amino acids were Essential, and seven were non-essential (Table 2). EAAs (Essential amino acids) such as threonine and arginine while NEAAs (Non-essential amino acids) proline was found absent in fish collected from the highly polluted site of the river. Overall, maximum amino acid con-tents/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 amino acid profile in different muscle zones (dorsal, ventral, ventricha and tail) of *Catla catla* were recorded among the fish samples harvested from different habitats. The contents of EAAs were found highest (0.1349±0.00023g/g) in ventricha muscle zone of *Catla catla* harvested from a less polluted site (upstream) of the river, closely followed by farmed fish (0.0983±0.00057g/g). The minimum concentration of EAAs (0.0249±0.0004g/g) were recorded in the ventral muscle zone of fish samples collected from the selected downstream location of the river. Contents of NEAAs were observed maximum in ventricha muscle zone of upstream wild fish (0.2091±0.0002g/g) closely followed by farmed fish (0.1846±0.0002g/g) and minimum in the tail muscle zone (0.0899±0.0002g/g) of fish harvested from highly polluted site of the river.

The greatest values EAAs were found in descending order in ventricha (0.0347±0.003g/g) > dorsal (0.0330±0.00020g/g) > tail (0.0280±0.0002g/g) > ventral (0.00249±0.0004g/g) muscle zones of *Catla catla* harvested from highly polluted site of river (Fig. 3). Maximum contents of EAAs in upstream wild fish muscle zones were observed in following order ventricha (0.1349±0.0001) > ventral (0.1318±0.0003) > dorsal (0.1312±0.0003) > tail (0.1305±0.0002) g/g. The highest values of EAA in muscle zones of farmed fish were noted in following sequence ventricha

(0.0983±0.0003g/g) > dorsal (0.0964±0.0004g/g) > ventral (0.0946±0.0003g/g) > tail (0.0913±0.0004g/g).

In the case of EAAs, the minimum phenylalanine concentration was determined in all muscle zones, especially in dorsal and ventricha muscle zones (0.0003±0.0002g/g) of fish samples harvested from downstream of the river. In the case of NEAAs, the minimum concentration of serine was recorded in all muscle zones, especially in the tail muscle zone (0.0028±0.0002g/g) of fish samples collected from a downstream area of the river.

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

Water Parameters	Highly Polluted Area	Less Polluted Area	Non Polluted Area	Desirable limits	Permissible limits
PH	11.70±0.071A	8.50±0.86B	7.62±0.11C	6.5-8.5	**, 6-10*
BOD (mg/L)	76.38±0.91A	44.66±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	324.40±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	206.20±3.19C	-	<100mg/L
Conductivity (mS/m)	3.222±0.031A	1.348±0.021B	0.336±0.010C	650µS/cm	1055µS/cm
Phenol (mg/L)	2.342±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 (Sb)	0.430±0.013A	0.002±0.000B	0.001±0.000B	0.01mg/L	**
Chromium(Cr)	0.470±0.003A	0.254±0.005B	0.036±0.002C	0.05mg/L	**, 1.0 mg/L*
Lead (Pb)	2.032±0.031A	0.232±0.013B	0.070±0.004C	0.05mg/L	**, 0.5 mg/L*
Zinc (Zn)	0.360±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)	1.630±0.022A	0.862±0.013B	0.051±0.004C	0.05mg/L	1.5mg/L, 1.0 mg/L*
Cadmium (Cd)	0.120±0.005A	0.070±0.006B	0.003±0.005C	0.01mg/L	**, 0.1 mg/L*
Mercury (Hg)	0.830±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

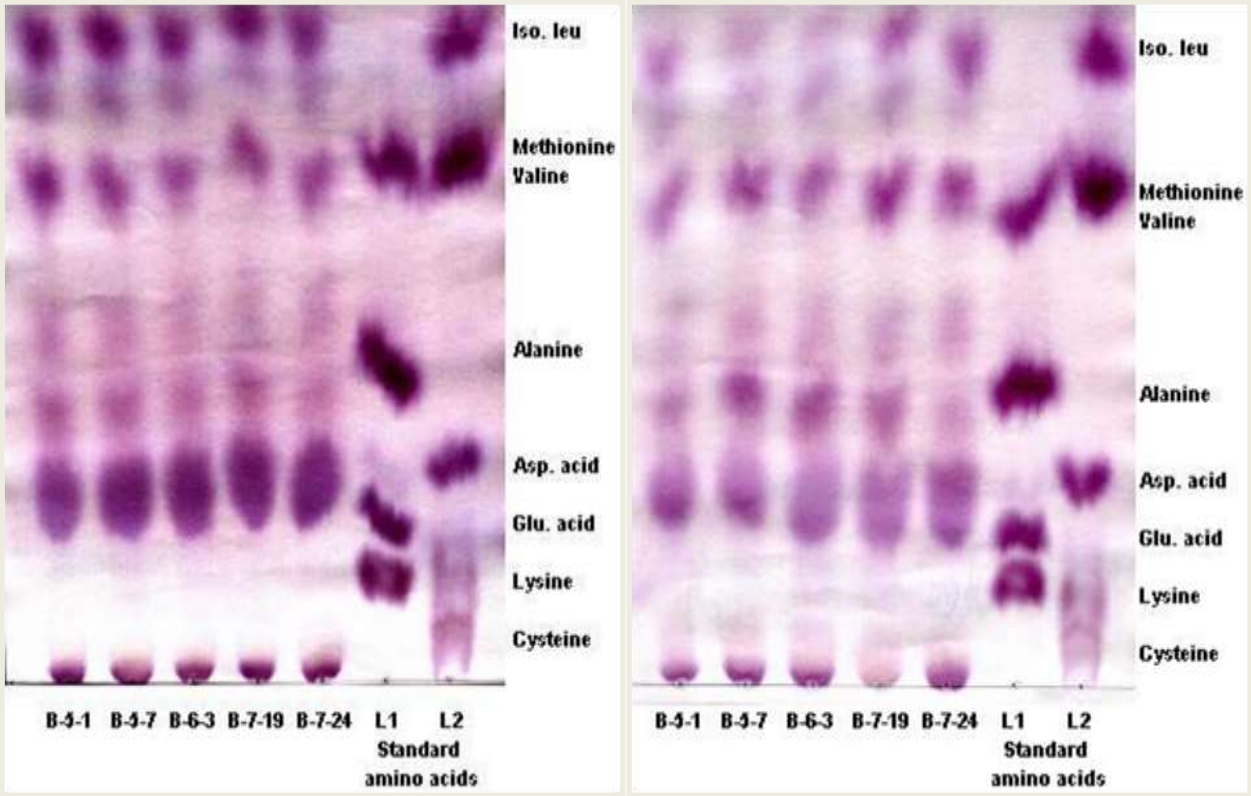
Table 2: Effect of different quality water on amino acid profile (g/g of dry meat) in different muscle zones of *Catla catla*

Amino acids	Polluted area*	Zone			
		Dorsal muscle	Ventral muscle	Ventricha muscle	Tail muscle
Valine	Highly	0.0080±0.0002d	0.0070±0.0002ef	0.0071±0.0002ef	0.0062±0.0002h
	Less	0.0086±0.0002ab	0.0084±0.0002bc	0.0087±0.0002a	0.0084±0.0002c
	Non-	0.0066±0.0002g	0.0072±0.0002e	0.0069±0.0002f	0.0063±0.0002h
Leucine	Highly	0.0040±0.0002e	0.0050±0.0002bc	0.0030±0.0002g	0.0030±0.0002g
	Less	0.0044±0.0002d	0.0045±0.0002d	0.0045±0.0002d	0.0037±0.0002f
	Non-	0.0052±0.0002b	0.0057±0.0002a	0.0050±0.0002c	0.0044±0.0001d
IsoLeucine	Highly	0.0032±0.0002g	0.0020±0.0002h	0.0041±0.0002e	0.0035±0.0002f
	Less	0.0088±0.0002b	0.0090±0.0002a	0.0092±0.0002a	0.0090±0.0002a
	Non-	0.0006±0.0002i	0.0006±0.0002i	0.0057±0.0002c	0.0047±0.0002d
Phenylalanine	Highly	0.0003±0.0002g	0.0007±0.0002f	0.0003±0.0002g	0.0007±0.0002f
	Less	0.0084±0.0001b	0.0089±0.0002a	0.0089±0.0002a	0.0081±0.0002cd
	Non-	0.0078±0.0002e	0.0082±0.0002bc	0.0079±0.0002de	0.0077±0.0002e
Methionine	Highly	0.0081±0.0002d	0.0031±0.0002g	0.0071±0.0002e	0.0050±0.0002f
	Less	0.0137±0.0002b	0.0138±0.0002b	0.0142±0.0002a	0.0138±0.0002b
	Non-	0.0104±0.0002c	0.0103±0.0002c	0.0105±0.0001c	0.0103±0.0002c
Threonine	Highly	ND	ND	ND	ND
	Less	0.0164±0.0003b	0.0159±0.0002d	0.0178±0.0002a	0.0161±0.0002c
	Non-	0.0102±0.0002e	0.0096±0.0002f	0.0097±0.0002f	0.0096±0.0002f
Lysine	Highly	0.0051±0.0002h	0.0021±0.0002j	0.0041±0.0002i	0.0052±0.0002h
	Less	0.0494±0.0002a	0.0487±0.0002c	0.0491±0.0002b	0.0495±0.0002a
	Non-	0.0373±0.0002d	0.0350±0.0001f	0.0355±0.0002e	0.0322±0.0002g
Arginine	Highly	ND	ND	ND	ND
	Less	0.0092±0.0002b	0.0097±0.0002a	0.0093±0.0002b	0.0094±0.0002b
	Non-	0.0085±0.0002c	0.0081±0.0002d	0.0076±0.0002e	0.0073±0.0002f
Histidine	Highly	0.0042±0.0002g	0.0050±0.0016f	0.0090±0.0002de	0.0044±0.0002fg
	Less	0.0123±0.0002b	0.0130±0.0002a	0.0131±0.0002a	0.0125±0.0003ab
	Non-	0.0097±0.0002c	0.0097±0.0002c	0.0095±0.0002cd	0.0087±0.0002e
Cystein	Highly	0.0276±0.0002f	0.0272±0.0002g	0.0271±0.0002g	0.0276±0.0002f
	Less	0.0303±0.0002d	0.0305±0.0002d	0.0313±0.0002c	0.0300±0.0002e
	Non-	0.0562±0.0002b	0.0562±0.0002b	0.0562±0.0002b	0.0565±0.0002a
ΣEAA	**Highly**	**0.0330±0.0002i**	**0.0249±0.0004k**	**0.0347±0.0003h**	**0.0280±0.0002j**
	Less	**0.1312±0.0003bc**	**0.1318±0.0003b**	**0.1349±0.0001a**	**0.1305±0.0002c**

	Non-	**0.0964±0.0004e**	**0.0946±0.0003f**	**0.0983±0.0003d**	**0.0913±0.0004g**
Aspartic Acids	Highly	0.0285±0.0002f	0.0221±0.0002h	0.0261±0.0002g	0.0213±0.0002i
	Less	0.0712±0.0002c	0.0717±0.0002b	0.0726±0.0002a	0.0712±0.0002c
	Non-	0.0513±0.0002e	0.0517±0.0001d	0.0513±0.0002e	0.0512±0.0002e
Tyrosine	Highly	0.0062±0.0002h	0.0066±0.0002g	0.0071±0.0002f	0.0062±0.0002h
	Less	0.0124±0.0002c	0.0133±0.0002b	0.0141±0.0002a	0.0122±0.0002c
	Non-	0.0076±0.0002d	0.0077±0.0001d	0.0074±0.0002e	0.0077±0.0002d
Serine	Highly	0.0007±0.0002gh	0.0007±0.0002gh	0.0005±0.0004h	0.0009±0.0002g
	Less	0.0028±0.0002f	0.0033±0.0002e	0.0037±0.0002d	0.0027±0.0002f
	Non-	0.0058±0.0002b	0.0053±0.0002c	0.0052±0.0002c	0.0060±0.0001a
Glycine	Highly	0.0023±0.0002g	0.0024±0.0002g	0.0016±0.0002h	0.0014±0.0002h
	Less	0.0053±0.0003b	0.0049±0.0002c	0.0056±0.0001a	0.0051±0.0003bc
	Non-	0.0043±0.0002d	0.0045±0.0002d	0.0038±0.0002e	0.0035±0.0002f
Proline	Highly	ND	ND	ND	ND
	Less	0.0034±0.0002ab	0.0035±0.0002a	0.0033±0.0002bc	0.0031±0.0002c
	Non-	0.0027±0.0002d	0.0027±0.0002d	0.0026±0.0002d	0.0021±0.0002e
Glutamic Acid	Highly	0.0284±0.0002g	0.0292±0.0002f	0.0298±0.0002e	0.0300±0.0002e
	Less	0.0492±0.0002c	0.0495±0.0002b	0.0498±0.0002a	0.0496±0.0003ab
	Non-	0.0392±0.0002d	0.0393±0.0003d	0.0393±0.0002d	0.0392±0.0001d
Alanine	Highly	0.0024±0.0002h	0.0028±0.0002g	0.0058±0.0001f	0.0025±0.0002h
	Less	0.0282±0.0002bc	0.0280±0.0002c	0.0287±0.0002a	0.0284±0.0002b
	Non-	0.0086±0.0001e	0.0087±0.0002e	0.0188±0.0002d	0.0088±0.0002e
ΣNEAA	**Highly**	**0.0961±0.0002h**	**0.0911±0.0002i**	**0.0981±0.0003g**	**0.0899±0.0002j**
	Less	**0.2028±0.0002c**	**0.2047±0.0003b**	**0.2091±0.0002a**	**0.2023±0.0004c**
	Non-	**0.1757±0.0003ef**	**0.1762±0.0002e**	**0.1846±0.0002d**	**0.1750±0.0003f**

Means sharing similar letters in a row or in a column within amino acid are statistically non-significant (P>0.05); * highly polluted area of river downstream to the entrance of CMD, less polluted area upstream to the entrance of CMD to river Chenab while third category is farmed.

FIGURE 3. Chromatograms exhibiting production of amino acids



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. The values of pH, total dissolved and suspended solids were higher that indirectly increased the values of BOD and COD (USEPA,2002), which not only made river water unsuitable for drinking but also for the growth of fish and ultimately led to the deterioration of quality of fish meat. Hanif et al. (2016) found the highest concentration of studied heavy metals in river Chenab at Trimmu Head, followed by other location, and related these results with the development of industries along the river. Different studies reported that heavy metals catalyze chemical reactions that produce reactive oxygen species, which lead to oxidative stress and harm

tissues and macromolecules (proteins and lipids) (Mahboob et al., 2011; Hussain et al., 2016; Sultana et al., 2019).

Our results showed that amino acid composition in the four muscle zone of *Catla catla* varied according to fish habitat change. The fish harvested from the downstream location of the river showed a significant ($P<0.05$) decrease in amino acid contents com-pared to upstream wild and farmed fish. Upstream wild fish have maximum amino acid contents closely followed by farm fish and minimum contents of amino acids in fish from downstream of the river. These findings are similar to Hussain et al. (2018) who reported in major carps (catla, rohu, and mrigal) harvested from a downstream area of the Chenab river had a decrease in amino acid profile than upstream wild and farmed fish. They related a decrease in amino acid composition in fish harvested from highly polluted river sites with the anthropogenic activities in the river. Arafa and Ali (2008) reported the concentration of different amino acids such as methionine, lysine, and cysteine that were significantly ($p<0.05$) decreased as compared to fish from pollution free environment. Significant differences ($p<0.05$) in amino acid contents were observed in fish species harvested from different polluted habitats (Das and Sahu, 2001). Similarly, under the current study, the amino acid contents were decreased in fish collected from a highly polluted environment. This finding was also in agreement with Ranbhare and Bakare (2012), who reported that elevated concentrations of heavy metals in fish harvested from freshwater caused a decrease in the biochemical composition (total glycogen, total lipids, and total protein contents) and ultimately leads to decrease in the production of fish.

Among EAAs, arginine, and threonine, proline, in the case of NEAAs was missing in all muscle zones of studied fish harvested from highly polluted sites of the river. The overall minimum EAAs were found in descending order in ventricha > dorsal > tail > ventral muscle zones of *Catla catla* harvested from the highly polluted river site com-pared to upstream wild and farmed fish. These findings were in line with results reported by Hussain et al. (2016) to find out significant differences ($p<0.05$) in the composition of amino acids of major carp *Cirrhinus mrigala* in different habitats. Many EAAs such as histidine, methionine, and phenylalanine were absent in studied fish collected from a polluted river area. Shakya et al. (2021) reported that the concentration of EAAs was relatively lower as only 43-44% of total amino acids in four studied taxa of insects harvested from a polluted river area compared NEAs. This change in their chemical composition is due to living in a stressful/polluted environment and ultimately decreasing the

supply of EAAs for consumers. Similarly, findings of Lane et al. (2019) related changes in biochemical composition based on variation in concentration of different amino acids, protein level, and physiological function of aquatic organisms that might be due to environmental pollution and heavy metal stress. Prakash et al. (2020) reported a significant decrease in protein content. They assumed an increase of free amino acids in the liver and muscle tissue of Mystus vittatus with proteolysis of protein under a stress-inducing toxic environment to fulfill energy demands. Hussain et al. (2016, 2018) also mentioned that the changes in the composition of amino acid profiles is related with the changes in the protein structure, disturbed regulation of different proteins (levels), and changes in the contents of free AAs in response to pollution. It has been reported that multiple factors such as diet composition, species, age, seasonal changes, and environmental conditions, and similarly, the condition of environmental change has shown that different environmental factors could cause a difference in biochemical composition and amino acid contents (Mol and Turan, 2008;El Shehawy et al., 2016).

The results of this study, particularly fish samples harvested from the polluted site, were in line with the study of Moses et al. (2018) reported that river in nearby industries is becoming toxic for aquatic biota with the reduced nutritional need of fish. They conducted a comparative study at different locations (dams and rivers) and reported decreased amino acids contents in tilapia fish inhabited in the river than in dams. They related increased pollution to gold miners' activities in the nearby river area. Our findings showed that maximum amino acid contents were in the different muscle of upstream wild fish than farmed fish, indicating that fish living in the natural environment have a choice of a variety of natural foods that most efficiently fulfills their energy requirements as compared to a single artificial diet that is often used in captivity. Rodrigues et al. (2022) also reported similar findings that EAAs were prominently higher for wild fish of larger size than farmed fish, and they related the difference in EAAs between wild and farmed due to natural and artificial diets. Our results corroborate with the findings of Wang et al. (2014). They reported that upstream wild fish have more amino acids contents than farmed fish. They also related the difference in amino acid profile not only with variation in the diet but also with differences in temperature, salinity, or storage time. Junao et al. (2018) reported similar findings that maximum concentrations of EAAs were recorded in the muscle of wild Japanese flounder (*Paralichthys olivaceus*) compared to farmed fish.

Our study showed that all studied amino acids were in low concentration in studied fish from the downstream location of the river compared with upstream wild and farmed fish. Similarly, Hussain et al. (2016) reported a pronounced effect of pollution that concentration of more commonly occurring amino acids such as aspartic acid, glutamic acid, alanine, and tyrosine was found in decreased concentrations in fish muscles collected from the polluted site of the river than collected fish from pollution free area of river and farm.

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

Conclusions

This study endorses the non-homogeneous composition of AAs contents in different muscle zones in *Catla catla*. Maximum EAAs and NEAAs were found in the ventricha 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. Whereas EAAs were recorded minimum in the ventral muscle zone of fish from downstream of the river showing that this region is most sensitive to pollution. The study recommends that upstream wild and farmed fish have good amino ac-id profiles 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 amino acid profile in different muscle zones of fish in response to surrounding water pollution to determine the muscle zone sensitivity to pollution. So, sustainability management re-quires 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 AAs 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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