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The Commercially Important Fish at the Risk of Toxic Elements; an empirical snapshot from river Chenab, Pakistan

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Abstract-The contents of trace elements (Al, Fe, S, Si, Cu, Cr, Sr, Mn and Zn) and macro-elements (Na, K, Ca, Mg, and P) in the muscle, liver and kidney of four freshwater fish species; (*Bagarius bagarius*, *Channa marulius*, *Mastacembelus armatus*, and *Notopterus notopterus*) from Head Trimmu (river Chenab) in Pakistan were examined through wet digestion method and samples were analyzed through Inductively Coupled Plasma-Optical Emission Spectroscopy. The study also evaluated the potential risks to human health associated with consuming these fishes by calculating estimated daily intake (EDI) and Target hazard quotient (THQ) values. The results showed that calcium was the most abundant macro-element in fish muscles, while phosphorus was observed to be the second-most abundant element, followed by potassium, sodium, and magnesium. The most frequent trace elements in all the four freshwater species were aluminium, sulphur and iron. The concentration of toxic trace elements was higher in the fish kidney and liver than in their muscles. The interactions between fish species and their tissues were only significant for Al, Mn, S, and Sr ($p<0.05$), according to a two-way ANOVA. However, maximum trace element concentrations in the muscles of different fish species were below allowable limits. The predicted EDI values for hazardous trace elements in four fish species were significantly lower than the consequent acceptable daily intakes, indicating that consumption of these fishes is safe. The THQ and total target hazard quotient (TTHQ) values for all four species were less than 1, indicating that there is no serious concern to consumers' health. In conclusion, this study showed that none of the four species from the Head Trimmu reservoir contained harmful trace elements that pose a serious risk to consumers' health. However, it is important to continue monitoring the levels of trace and macro-elements in fishes to ensure that they remain safe for consumption.

Index Terms-Estimated Daily Intake, Fish, Head Trimmu, Macro-elements, Risk Assessment, Trace elements

I. INTRODUCTION

Fish is a vital element of a balanced diet as it is a good supply of proteins, vitamins, minerals, and omega-3 fatty acids. The consumption of fish has been rising quickly worldwide [1, 2]. Fish, however, can ingest some environmental toxins, like trace elements [3]. Health issues might result from consuming too much seafood contaminated with trace substances [4, 5]. Macro-elements are crucial for the maintenance of human health [5]. Potassium and sodium regulate the osmo-regulation within the body of organisms and trigger a number of enzymes. Magnesium activates about 300 enzymes within the organisms. Calcium is necessary for healthy bones. Phosphorus is necessary for building and storing energy in addition to maintaining healthy bones [6, 7].

Trace element-related environmental pollution has sparked considerable concern throughout the planet [8]. Essential trace elements include zinc (Zn), iron (Fe), manganese (Mn), and chromium (Cr), while non-essential trace elements include lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg) [9]. Toxic effects could occur if organisms are exposed to certain components too frequently [10]. Even at low quantities, prolonged contact to non-essential trace elements is harmful to organisms [11, 12]. In various fish organs or tissues, toxic trace elements (TTES) levels can exhibit considerable variation. Fish liver and kidneys have higher TTE values than muscles, according to numerous research studies [13, 14]. Fish are, therefore, trustworthy bio-indicators for TTE contamination in aquatic environments [15]. Numerous studies have been conducted in recent years to evaluate potential health concerns associated with eating fish that has been exposed to TTEs [16-18].

Trace elements can be a reason of both cancer and non-cancer health issues in people. Consequently multiple methods for determining the risks to human health caused due to TTEs exist [19], [20]. Target hazard quotient (THQ) and total target hazard quotient (TTHQ) or hazard index (HI) are frequently used to assess possible dangers to non-carcinogenic physical condition [21]. Many researchers have shown the application and reliability of numerous risk assessment methodologies [20, 22-24]. In Punjab, Pakistan, River Chenab is a significant trans-boundary river. On the Chenab River, Head Trimmu is one of the significant heads. Reservoir contamination is mostly caused by the discharge of household and industrial wastewater, farming practices, fish farms, and inflowing rivers [15, 23].

The central goals of this study were to measure the concentrations of macro-elements and TTES in the liver, muscle tissue and kidney of four freshwater fish species from Trimmu reservoir, to compare the concentrations of TTEs and macro-elements (MEs) in native fish species, and to evaluate the risks to both carcinogenic and non-carcinogenic health from consuming fish.

II. MATERIALS AND PROCEDURES

A. *Collection of samples and their processing*

In the current study, four fish species from the Sisoridae, Channidae, Notopteridae and Mastacembelidae families collected from Head Trimmu were taken into consideration: Fauji khagga (*Bagarius bagarius*), saul (*Channa marulius*), parri (*Notopterus notopterus*) and baam (*Mastacembelus armatus*), respectively in March 2021. After measuring their live length and weight, the fish specimen were sealed in polyethylene bags, and shipped on ice to the Ecology and Evolutionary Biology Laboratory (Institute of Zoology, University of the Punjab), Pakistan. Carefully separated tissues were then lyophilized to achieve stable weight. The lyophilized samples were homogeneously powdered after grinding, and they were stored in plastic bags at 20°C until their digestion and analysis.

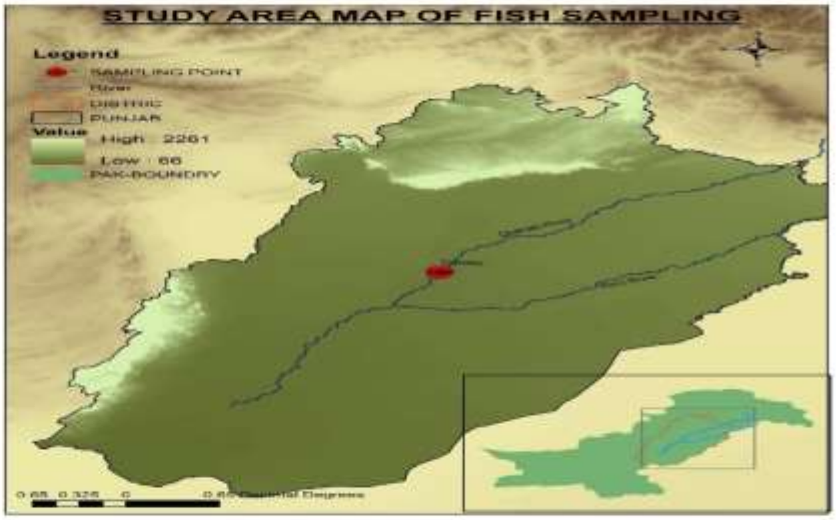


Fig 1: Map showing study area and sampling site

B. Sample digestion and analysis of trace elements

A sample of fish kidney, muscle and liver (weighing between 1.5 and 2.0g) was placed into each digesting vessel, along with 20ml HNO_3 and 10ml $HClO_4$. Acid digestion technique was used to digest the mixture. Then the samples were chilled to room temperature and put into 50ml polypropylene tubes after being diluted with ultrapure water. To evaluate the concentrations of hazardous trace elements (Fe, Cr, Cu, Al, Sr, S, Si, Mn, Zn) and macro-elements (Na, Ca, K, Mg, and P), an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) was used for the digested samples. The approximate values from each sample were utilized to analyze the data. Samples for TTEs and MEs were digested and then analyzed to ensure that the method was accurate.

C. Statistical evaluations

The mean values were used to show the concentrations of MEs and potentially dangerous trace in various freshwater fish species. To assess any differences in the concentration of these MEs and TTEs across the different fish species (Fauji khagga, Saul, Parri, and Baam) and tissues of these fishes (liver, kidney and muscle), two-way ANOVA ($p<0.05$) was performed. Statistical analysis was conducted using SPSS 16.

D. Risk evaluation for human health

Pakistani people consume a significant amount of fish in their diet. Here, we assumed that if the native people eat the fish, since the muscle is the only eatable component of fish for humans; the risks of this assumption are obliged to be considered. Calculations were made to conclude the lifetime carcinogenic risk (CR), THQ, and estimated daily intake (EDI) of MEs & TTEs for adults who consume fish (Table 11).

III. RESULTS

A. *Bio-concentration of macro-elements*

In the current study, the highest concentration of Ca (64.5±0.79µg/ml)) was found in the muscle of *N. notopterus* (Table 3), while the lowest concentration of Ca (3.8±1.37µg/ml) was recorded in the liver of *C. marulius* (Table 2). The muscle of *C. marulius* exhibited maximum accumulation rate of K (35.1±3.84µg/ml), whereas minimum level of K (3.05±1.39µg/ml) was detected in the kidney of *C. marulius* (Table 2). Mg was found to accumulate in the highest concentration (7.77±1.13 µg/ml) in the kidney of *N. notopterus* (Table 3), while its lowest concentration (1.03±0.53µg/ml) was detected in the kidney of *C. marulius* (Table 2).

The concentration of Na was recorded highest (20.6±3.63µg/ml) in the muscle of *C. marulius* (Table 2) and lowest level (10.9±0.53µg/ml) of Na was found in the liver of *N. notopterus* (Table 3). The maximum concentration of P (42.8±0.70µg/ml) was reported in the muscle of *N. notopterus* (Table 3), whereas minimum level of P (7.57±0.79µg/ml) was detected in the kidney of *C. marulius* (Table 2). There were significant variations between concentration of macro-elements in kidney, muscle and liver of studied fish species ($p < 0.05$) (Table 10).

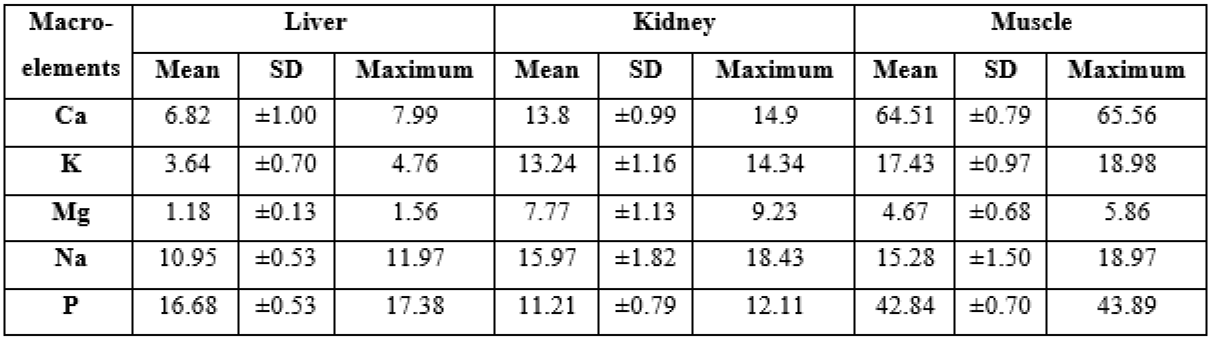
Table 1 Concentration (µg/ml) of macro-elements in various tissues of *B. bagarius* from the Head Trimmu

Macro-elements	Liver			Kidney			Muscle		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Ca	4.66	±1.562	7.42	43.89	±4.158	51.1	6.68	±1.866	9.73
K	3.97	±1.264	5.96	4.678	±1.327	6.82	12.9	±2.626	16.7
Mg	1.88	±0.754	2.78	1.444	±0.760	2.78	2.64	±1.193	4.53
Na	11.78	±2.894	15.83	11.35	±2.115	14.51	16.07	±3.618	22.63
P	24.34	±3.617	30.52	29.43	±2.236	32.6	13.72	±3.708	19.74

Table 2 Concentration (µg/ml) of macro-elements in various tissues of *C. marulius* from the Head Trimmu

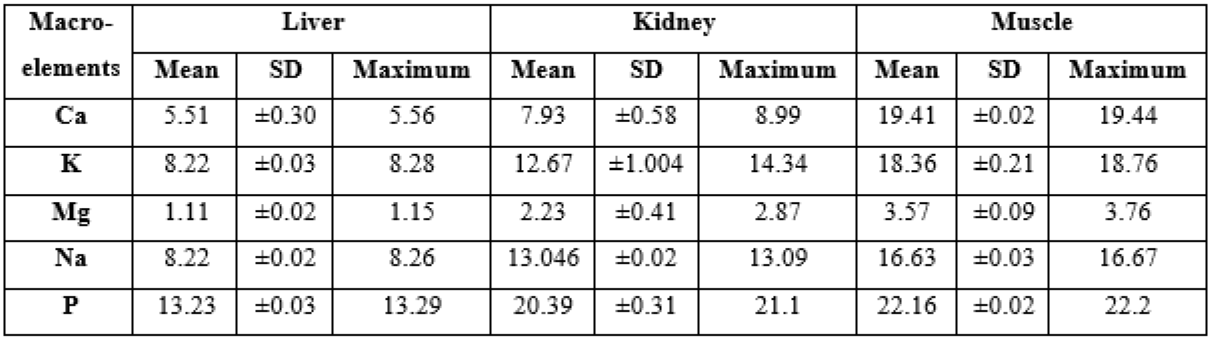
Macro-elements	Liver			Kidney			Muscle		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Ca	3.8	±1.378	5.9	5.1	±2.139	8.7	17.7	±2.632	23.36
K	8.97	±2.556	13.5	3.05	±1.399	5.62	35.1	±3.847	41.66
Mg	1.62	±0.739	2.74	1.03	±0.536	1.57	5.71	±2.256	9.34
Na	13.0	±3.221	17.6	11.2	±2.988	15.6	20.67	±3.633	25.67
P	17.9	±2.537	23.4	7.57	±0.791	9.0	28.17	±2.235	31.51

Table 3 Concentration (µg/ml) of macro-elements in various tissues of *N. notopterus* from the Head Trimmu



Macro-elements	Liver			Kidney			Muscle		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Ca	6.82	±1.00	7.99	13.8	±0.99	14.9	64.51	±0.79	65.56
K	3.64	±0.70	4.76	13.24	±1.16	14.34	17.43	±0.97	18.98
Mg	1.18	±0.13	1.56	7.77	±1.13	9.23	4.67	±0.68	5.86
Na	10.95	±0.53	11.97	15.97	±1.82	18.43	15.28	±1.50	18.97
P	16.68	±0.53	17.38	11.21	±0.79	12.11	42.84	±0.70	43.89

Table 4 Concentration (µg/ml) of macro-elements in various tissues of *M. armatus* from the Head Trimmu



Macro-elements	Liver			Kidney			Muscle		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Ca	5.51	±0.30	5.56	7.93	±0.58	8.99	19.41	±0.02	19.44
K	8.22	±0.03	8.28	12.67	±1.004	14.34	18.36	±0.21	18.76
Mg	1.11	±0.02	1.15	2.23	±0.41	2.87	3.57	±0.09	3.76
Na	8.22	±0.02	8.26	13.046	±0.02	13.09	16.63	±0.03	16.67
P	13.23	±0.03	13.29	20.39	±0.31	21.1	22.16	±0.02	22.2

B. Bio-concentration of trace elements

In the current study, the highest concentration of Al (395.1±3.81µg/ml)) was found in the kidney of *M. armatus* (Table 8), while the lowest concentration of Al (0.34±0.01µg/ml) was recorded in the liver of *N. notopterus* (Table 7). The concentration of Al showed significant differences between various organs of fish species ($p < 0.05$) (Table 9). The kidney of *M. armatus* was found to contain the highest concentration of Cr (0.07±0.01 µg/ml) (Table 8), while the lowest accumulation pattern (0.02±0.01 µg/ml) was recorded in the kidney and liver of *C. marulius* and *N. notopterus*, respectively (Table 6, 7). The Cr concentration did not show significant differences between the muscles of studied fish species ($p>0.05$) (Table 9).

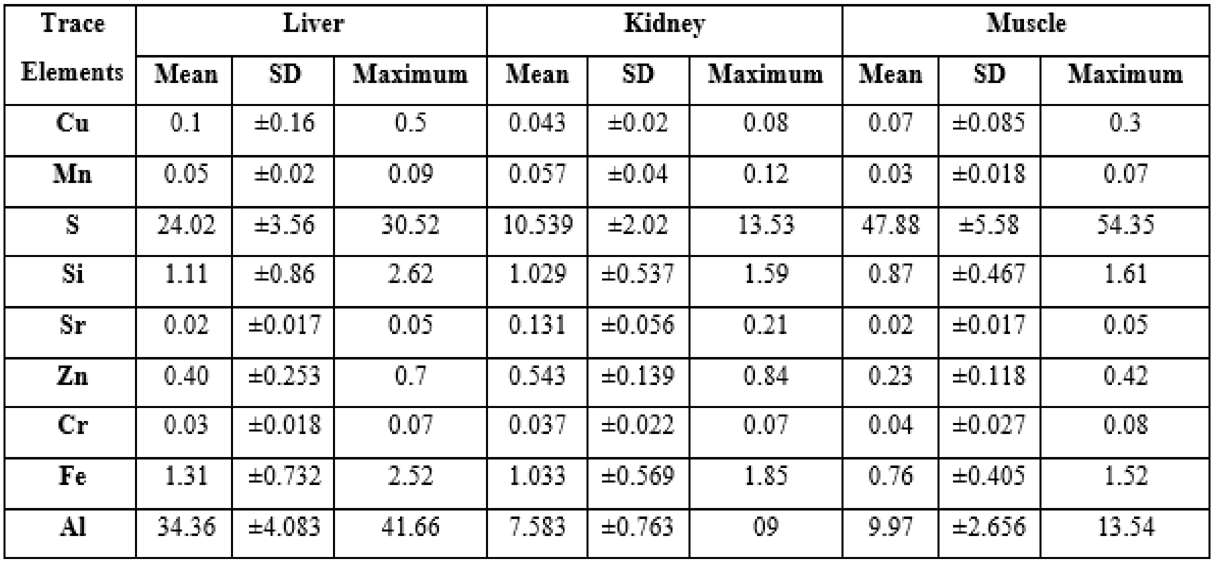
The highest concentration of Cu (0.16±.01 µg/ml) was detected in kidney of *M. armatus* (Table 8), while the lowest accumulation rate of Cu (0.04±0.02 µg/ml) was found in kidney of *B. bagarius* (Table 5). The level of Cu varied significantly in kidney ($p<0.05$), while no significant variations were recorded in Cu concentration between the liver and muscles of fish species ($p>0.05$) (Table 9). The kidney of *N. notopterus* had accumulated maximum level of Fe (23.48±1.43 µg/ml), whereas minimum concentration (0.63±0.01 µg/ml) was recorded in the muscle of *N. notopterus* (Table 7). The concentration of Fe did not show significant differences in the muscle ($p>0.05$), whereas Fe concentration varied significantly in the kidney and liver of fish species ($p<0.05$) (Table 9).

The kidney of *M. armatus* exhibited maximum accumulation rate of Mn (0.38±0.10µg/ml) (Table 8), whereas minimum level of Mn (0.01±0.01µg/ml) was detected in the kidney of *C. marulius* (Table 6). Statistically significant variations were found between the accumulation rate of Mn in various organs of fish ($p<0.05$) (Table 9). The concentration of S was recorded highest (70.8±5.69µg/ml) in the muscle of *C. marulius* (Table 6) and the lowest level (10.5±2.02µg/ml) of S was found in the kidney of *B. bagarius* (Table 5). The concentration of S varied significantly between organs of fish species ($p<0.05$) (Table 9). The highest level of Si (5.05±0.01µg/ml) was reported in the kidney of *M. armatus* (Table 8), while the lowest level of Si (0.69±0.01µg/ml) was detected in the kidney of *N. notopterus* (Table 7). The significant differences were recorded between the accumulation rate of Si in the kidney and liver ($p<0.05$), while Si concentration did not vary significantly between the muscle ($p>0.05$) (Table 9).

The liver of *N. notopterus* exhibited maximum accumulation rate of Sr (0.27±0.01µg/ml) (Table 7), whereas minimum level of Sr (0.01±0.01µg/ml) was detected in the liver of *C. marulius* (Table 6).There were significant interactions between tissues and species for Sr concentration ($p<0.05$) (Table 9). The highest concentration of Zn (0.87±0.02µg/ml) was reported in the kidney of *M. armatus* (Table 8) however, the lowest concentration of Zn (0.16±0.03µg/ml) was detected in the kidney of *C. marulius* (Table 6). The mean concentration of Zn varied significantly between the kidney ($p<0.05$) whereas significant variations were not recorded for the concentration of Zn in muscles and liver of studied fish species ($p>0.05$) (Table 9).

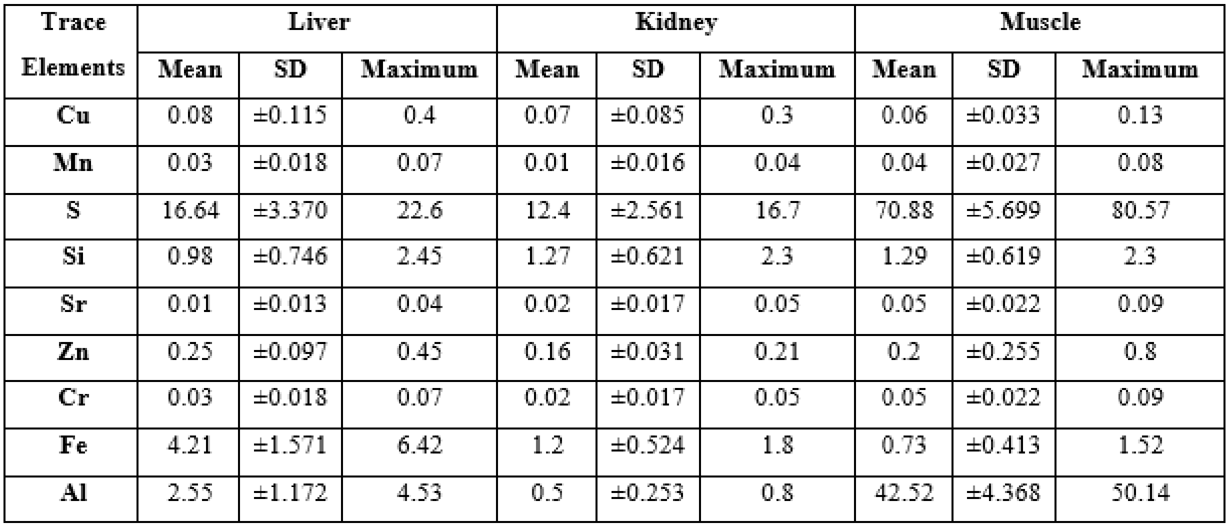
The accumulation of trace elements in the liver, muscle and kidney of *C. marulius* was found in the following order: Al>S>Fe>Si>Zn>Cu>Cr>=Mn>Sr, S>Si>Fe>Al>Zn>Cu>Cr=Sr>Mn, S>Si>Fe>Al>Zn>Cu, Cr=Sr>Mn and S>Al>Si>Fe>Zn>Cu>Cr=Sr>Mn, respectively (Table 6). The accumulation pattern of trace elements in muscle, liver and kidney of *B. bagarius* was recorded in the following sequence: S>Al>S>Fe>Zn>Cu>Cr>Mn>Sr, Al>S>Fe>Si>Zn>Cu>Mn>Cr>Sr and S>Al>Fe>Si>Zn>Sr>Mn>Cu>Cr, respectively (Table 5). The level of trace metals in the kidney, liver and muscle of *N. notopterus* followed this order: Al>S>Fe>Si>Zn>Mn>Cu>Cr>Sr, S>Fe>Si>Al>Zn>Sr>Cu>Mn>Cr and S>Al>Si>Fe>Zn>Sr>Cu>Mn>Cr, respectively (Table 7). Trace elements in liver, kidney and muscle of *M. armatus* were recorded in following pattern: Al>S>Fe>Si>Zn>Mn>Cu>Cr>Sr, Al>S>Fe>Si>Zn>Mn>Cu>Cr>Sr and S>Al>Si>Fe>Zn>Cu>Cr=Sr>Mn, respectively (Table 8).

Table 5 Concentration (µg/ml) of trace elements in various tissues of *B. bagarius* from the Head Trimmu



Trace Elements	Liver			Kidney			Muscle		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Cu	0.1	±0.16	0.5	0.043	±0.02	0.08	0.07	±0.085	0.3
Mn	0.05	±0.02	0.09	0.057	±0.04	0.12	0.03	±0.018	0.07
S	24.02	±3.56	30.52	10.539	±2.02	13.53	47.88	±5.58	54.35
Si	1.11	±0.86	2.62	1.029	±0.537	1.59	0.87	±0.467	1.61
Sr	0.02	±0.017	0.05	0.131	±0.056	0.21	0.02	±0.017	0.05
Zn	0.40	±0.253	0.7	0.543	±0.139	0.84	0.23	±0.118	0.42
Cr	0.03	±0.018	0.07	0.037	±0.022	0.07	0.04	±0.027	0.08
Fe	1.31	±0.732	2.52	1.033	±0.569	1.85	0.76	±0.405	1.52
Al	34.36	±4.083	41.66	7.583	±0.763	09	9.97	±2.656	13.54

Table 6 Concentration (µg/ml) of trace elements in various tissues of *C. marulius* from the Head Trimmu



Trace Elements	Liver			Kidney			Muscle		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Cu	0.08	±0.115	0.4	0.07	±0.085	0.3	0.06	±0.033	0.13
Mn	0.03	±0.018	0.07	0.01	±0.016	0.04	0.04	±0.027	0.08
S	16.64	±3.370	22.6	12.4	±2.561	16.7	70.88	±5.699	80.57
Si	0.98	±0.746	2.45	1.27	±0.621	2.3	1.29	±0.619	2.3
Sr	0.01	±0.013	0.04	0.02	±0.017	0.05	0.05	±0.022	0.09
Zn	0.25	±0.097	0.45	0.16	±0.031	0.21	0.2	±0.255	0.8
Cr	0.03	±0.018	0.07	0.02	±0.017	0.05	0.05	±0.022	0.09
Fe	4.21	±1.571	6.42	1.2	±0.524	1.8	0.73	±0.413	1.52
Al	2.55	±1.172	4.53	0.5	±0.253	0.8	42.52	±4.368	50.14

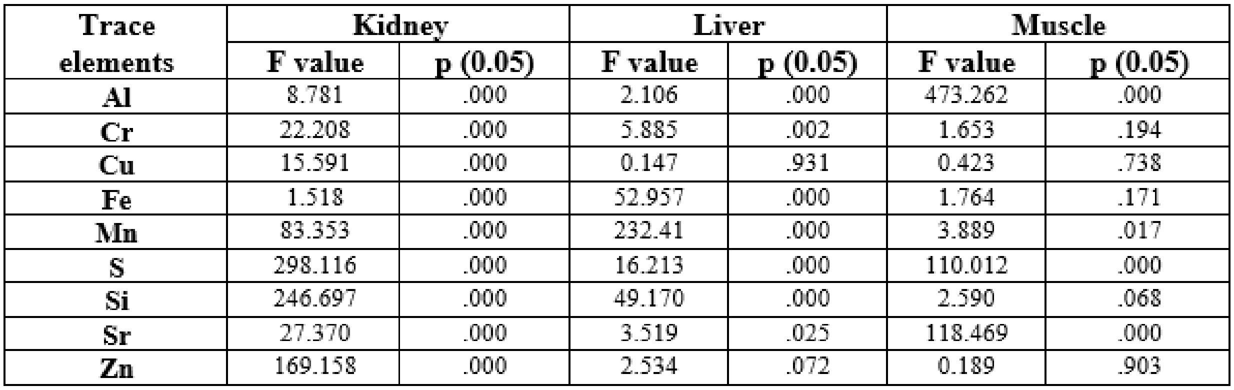
Table 7 Concentration (µg/ml) of trace elements in various tissues of *N. notopterus* from the Head Trimmu

Trace Elements	Liver			Kidney			Muscle		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Cu	0.11	±0.01	0.14	0.13	±0.01	0.15	0.06	±0.01	0.08
Mn	0.06	±0.01	0.09	0.22	±0.01	0.25	0.05	±0.01	0.08
S	18.25	±0.75	19.33	28.9	±0.80	30.56	41.55	±1.03	43.23
Si	1.01	±0.01	1.04	0.69	±0.01	0.71	0.908	±0.05	0.99
Sr	0.27	±0.01	0.04	0.05	±0.01	0.07	0.15	±0.12	0.17
Zn	0.33	±0.01	0.31	0.38	±0.01	0.4	0.25	±0.108	0.4
Cr	0.02	±0.01	0.04	0.068	±0.01	0.09	0.037	±0.01	0.05
Fe	6.23	±0.99	7.76	23.48	±1.43	20.99	0.63	±0.01	0.6
Al	0.34	±0.01	0.37	33.1	±1.20	35.9	11.61	±0.60	12.5

Table 8 Concentration (µg/ml) of trace elements in various tissues of *M. armatus* from the Head Trimmu

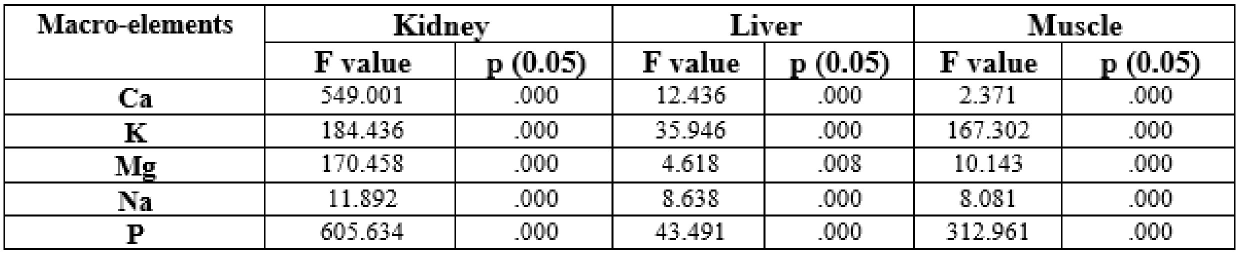
Trace Elements	Liver			Kidney			Muscle		
	Mean	SD	Maximum	Mean	SD	Maximum	Mean	SD	Maximum
Cu	0.10	±0.09	0.3	0.16	±0.01	0.18	0.08	±0.009	0.09
Mn	0.22	±0.01	0.25	0.38	±0.10	0.56	0.030	±0.01	0.06
S	20.13	±0.53	20.9	27.14	±1.05	28.32	44.62	±0.04	44.69
Si	3.56	±0.07	3.67	5.05	±0.01	5.07	1.15	±0.11	1.4
Sr	0.02	±0.01	0.05	0.029	±0.01	0.06	0.05	±0.01	0.07
Zn	0.39	±0.02	0.43	0.87	±0.02	0.94	0.23	0.02	0.19
Cr	0.05	±0.01	0.08	0.07	±0.01	0.09	0.05	±0.01	0.08
Fe	6.11	±0.02	6.14	6.14	±0.54	7.12	0.92	±0.01	0.95
Al	369.3	±6.54	387	395.1	±3.81	399	2.43	±0.12	2.6

Table 9 Two-way ANOVA for comparison of trace elements concentration in different organs of fish species



Trace elements	Kidney		Liver		Muscle	
	F value	p (0.05)	F value	p (0.05)	F value	p (0.05)
Al	8.781	.000	2.106	.000	473.262	.000
Cr	22.208	.000	5.885	.002	1.653	.194
Cu	15.591	.000	0.147	.931	0.423	.738
Fe	1.518	.000	52.957	.000	1.764	.171
Mn	83.353	.000	232.41	.000	3.889	.017
S	298.116	.000	16.213	.000	110.012	.000
Si	246.697	.000	49.170	.000	2.590	.068
Sr	27.370	.000	3.519	.025	118.469	.000
Zn	169.158	.000	2.534	.072	0.189	.903

Table 10 Two-way ANOVA for comparison of macro-elements concentration in different organs of fish species



Macro-elements	Kidney		Liver		Muscle	
	F value	p (0.05)	F value	p (0.05)	F value	p (0.05)
Ca	549.001	.000	12.436	.000	2.371	.000
K	184.436	.000	35.946	.000	167.302	.000
Mg	170.458	.000	4.618	.008	10.143	.000
Na	11.892	.000	8.638	.000	8.081	.000
P	605.634	.000	43.491	.000	312.961	.000

C. Estimated Daily Intake (EDI)

Heavy metals have a tendency to accumulate in a variety of aquatic creatures' organs, particularly in fish, and when consumed by humans, they can pose a serious health risk [25]. In order to determine if the metal concentrations discovered in fish samples from the Head Trimmu were safe for typical human consumption, the daily intake of some selected trace metals was estimated and compared with the prescribed values (Table 11). Since humans eat the majority of the fish's muscle, this study simply took that into account. For adults of average age, the highest recorded EDI value was 8.40E-05 for *M. armatus* (mg/day/person) found in Fe, and the lowest value was discovered in *N. notopterus* (0.000005 mg/day/person) found in Cu (Table 11). The main route of exposure was through the consumption of aquatic foods, as opposed to potential risk factors including inhalation and direct skin contact [26]. The outcomes for ingesting in the research area fell below the recommended daily allowance (RDA). For the targeted groups of people, EDIs that were lower than RDA suggested a potential lower health effect. However, drawing conclusions about "acceptable limit" and "unacceptable limit" based on doses below RDA/Rfd was not a stable measurement technique [27, 28].

The levels of TTEs discovered just in the muscle of fish species were used for the risk assessment methodology in the current study, since people in Pakistan do not consume other fish components. The EDI of TTEs for adults was determined using the following equation [20,22, 29]

$$\text{EDI} = \text{MC}\times\text{DC}/1000\times\text{BW}\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.\ (1)$$

DC is the daily fish consumption average (g/day). Adults in Pakistan ate 2kg (1.9kg more precisely) of fish per capita per year [2], on average 5.479g per day. BW is the normal adult body weight 60kg, and MC is the amount of toxic trace elements (TTE) present in fish muscle (mg/kg wet weight).

D. THQ and HI

The USEPA [30] THQ and HI criteria, which compares the quantity of a pollutant consumed with a set reference dose, have been frequently utilized in the evaluation of the danger posed by the metals in contaminated foods [31]. The THQ value has additionally been acknowledged as one of the suitable measures for the risk assessment of metals linked to consuming contaminated seafood [32]. USEPA [33] recommends the threshold limit for THQ to be 1. The results showed that for an average group of humans, the mean THQ of all the species was below 1 (Table 11). None of the metals in any of the species was above the threshold limit, proving that eating these species does not significantly increase the risk of exposure to metals to humans. THQ of Fe in *B. bagarius* showed the greatest value (9.9E-05), while THQ of Fe in *N. notopterus* showed the lowest THQ of 0.00008 (Table 11).

The results highlighted the need for HI evaluation, where exposures that exceeded HI unit thresholds defined the alarming situation of health risk for local consumers [34, 35]. The investigated HI fell within the advised range. When HI is greater than 1, the metals are poisonous and dangerous for human health [32].

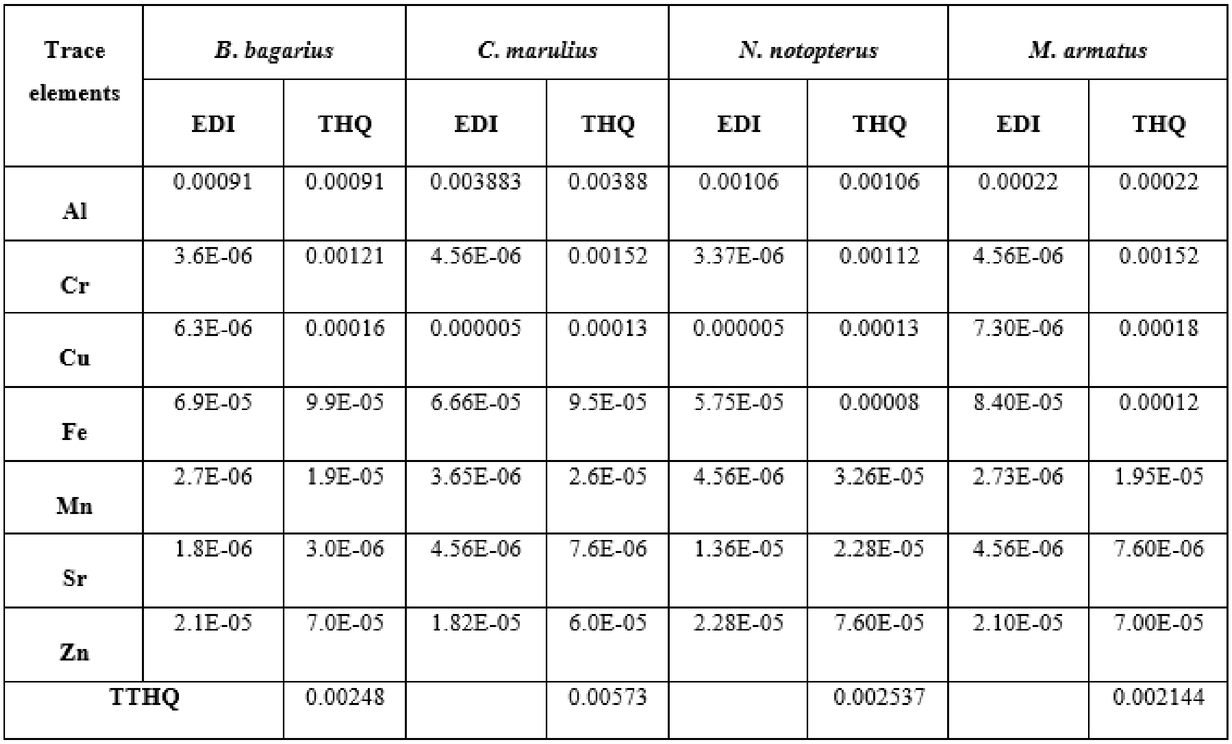
The average HI values in the current study were below the threshold value for all fish species, indicating that ingesting these fishes does not pose a significant risk to human health. THQ and HI are not regarded as a direct measurement of risk concern, however, because there is no clear dose association [30].

THQ) and TTHQ were employed to assess the non-cancer health risks connected with consuming different fish species (or hazard index). If the THQ and TTHQ readings are greater than 1, there may be a risk for adverse non-cancer health impacts to manifest. If the numbers are lower, no effects on health conditions other than cancer are expected [21]. The following equations were used to determine the THQ and TTHQ [30].

$$\mathrm{THQ} = \mathrm{EDI} / \mathrm{RFD} \quad (2)$$

$$\mathrm{TTHQ} = \mathrm{THQ}(\mathrm{Al}) + \mathrm{THQ}(\mathrm{Cr}) + \mathrm{THQ}(\mathrm{Fe}) + \mathrm{THQ}(\mathrm{S}) + \mathrm{THQ}(\mathrm{Cu}) + \mathrm{THQ}(\mathrm{Mn}) + \mathrm{THQ}(\mathrm{Si}) + \mathrm{THQ}(\mathrm{Sr}) + \mathrm{THQ}(\mathrm{Zn}) \quad (3)$$

Table 11 Estimated daily intake (EDI), Target hazard quotient (THQ) and Total target hazard quotient (TTHQ) from the consumption of fish muscle



Trace elements	*B. bagarius*		*C. marulius*		*N. notopterus*		*M. armatus*	
	EDI	THQ	EDI	THQ	EDI	THQ	EDI	THQ
Al	0.00091	0.00091	0.003883	0.00388	0.00106	0.00106	0.00022	0.00022
Cr	3.6E-06	0.00121	4.56E-06	0.00152	3.37E-06	0.00112	4.56E-06	0.00152
Cu	6.3E-06	0.00016	0.000005	0.00013	0.000005	0.00013	7.30E-06	0.00018
Fe	6.9E-05	9.9E-05	6.66E-05	9.5E-05	5.75E-05	0.00008	8.40E-05	0.00012
Mn	2.7E-06	1.9E-05	3.65E-06	2.6E-05	4.56E-06	3.26E-05	2.73E-06	1.95E-05
Sr	1.8E-06	3.0E-06	4.56E-06	7.6E-06	1.36E-05	2.28E-05	4.56E-06	7.60E-06
Zn	2.1E-05	7.0E-05	1.82E-05	6.0E-05	2.28E-05	7.60E-05	2.10E-05	7.00E-05
TTHQ		0.00248		0.00573		0.002537		0.002144

IV. DISCUSSION

A. *Bio-accumulation of macro-elements in fish tissues*

The results indicated that all the macro-elements under study had collected in the liver, kidney and muscles of four freshwater fish species; *N. notopterus, M. armatus, B. bagarius, C. marulius*. Regarding fish species the overall mean concentration of macro-elements was observed in the following pattern: Ca>P>K>Na>Mg (Table 1, 2, 3, 4).

Regarding fish organs i.e., kidney, liver and muscle of *B. Bagarius* contain the MEs in the following order Ca>P>Na>K>Mg, P>Na>Ca>K>Mg, Na>P>K>Ca>Mg (Table 1) while the order observed in *C. marulius* was as Na>P>Ca>K>Mg, P>Na>K>Ca>Mg, K>P>Na>Ca>Mg (Table 2). In *N. notopterus*, kidney, liver and muscle tissue contained macro-elements in the following manner as Na>Ca>K>P>Mg, P>Na>Ca>K>Mg and Ca>P>K>Na>Mg respectively, as shown in Table 3 & the order in kidney and liver of *M. armatus* was observed as P>Na>K>Ca>Mg while muscle showed the order as P>Ca>K>Na>Mg (Table 4).

In the liver of all four fish species, P was the most frequent macro-element, followed by Na in the kidneys of *C. marulius* and *N. notopterus*. K and P were also shown to be the most prevalent macro-elements in the muscles of different fish species from the Mazurian Great Lakes, including bream, pike, Eurasian perch, and Burbot (Poland). Additionally, Njinkoue et al. [36] discovered that K and P concentrations in the edible parts of two freshwater fish species (*Pseudo tolithus elongates* and *P*. *typus*) from the Cameroonian shore were significantly high than Ca, Na and Mg, which is consistent with our findings that K, P, and Na are present in muscles in greater amounts than Ca and Mg.

Muscle function benefits from sodium. Too much Na consumption can cause disorders related to high blood pressure [6]. However, the ratio of Na to K in diet has a stronger, more beneficial relationship with high blood pressure in younger people than either Na or K alone [37].

Macro-elements Mg, Ca, K, and Na are necessary for human health, yet these elements are toxic in excess amounts as studied by Walker et al. [38]. Nofa [39] discovered that the large levels of P found in fish muscles are only partially absorbed by humans due to low levels of the P-assimilating minerals; Ca and Mg. Compared to the bones, fins, and scales of a fish, the muscle is largely composed of water and contains fewer minerals [40]. Fish nutritional value and macronutrient composition vary depending on the species, size, age, sexual maturity, water quality, feed, season, and potential contaminants [41-45].

Ca is crucial for the development of bones. Fish is a well-known good supply of this macro-element, and little fishes are thought to have more of this macro-element [46, 47].

A balance between K (high level) and Na (low level) is required for optimal human nutrition, according to studies in the field [48, 49]. Mg is essential for the growth of bones and new cells, the activation of vitamin B, the formation of blood clots, the metabolism of energy, and the synthesis of RNA and DNA in cell membranes[50, 51]. According to Lall [52], fish are poor sources of Mg. This was supported by a recent study, when it was discovered that all four species' organs contained the least amount of Mg.

The inclusion of protein, omega-3, vitamins, and an ideal mineral composition in fish may provide health benefits for humans. In conclusion, the findings of this study can be applied to research on food composition and human nutrition.

B. Trace elements

This study revealed that certain trace elements gathered in the kidney, muscle and liver of four freshwater fish species; *N. notopterus, M. armatus, B. bagarius, C. marulius.* With regard to fish species, the following pattern was seen in the total mean concentration of trace elements: kidney of *C. marulius* comprise S>Al>Si>Fe>Zn>Cu>Cr=Sr>Mn, while the same order in *B. bagarius* was observed as S>Al>Fe>Si>Zn>Sr>Mn>Cu>Cr, in *N. notopterus* the order of the trace elements in kidney is Al>S>Fe>Si>Zn>Mn>Cu>Cr>Sr and in *M. armatus* same order was observed as Al>S>Fe>Si>Zn>Mn>Cu>Cr> Sr.

Comparable trace elements deposition trends were also discovered in *L. rohita* collected by Mahmood et al. [53] from the river Ravi. Furthermore, according to Qadir et al. [54], the ranking order of metal accumulation in *L. rohita* was Fe>Zn>Cr>Pb>Ni>Cu>Cd. Even though Cr, Ni, and Cu only play a minor role in metabolic processes and are only required in very small levels, some fish species also had significant amounts of these metals.

In the three species under study (*C. marulius*, *B. Bagarius* and *M. armatus*), the pattern of accumulation of the metals Al>S>Fe>Si>Zn was the same while the pattern was relatively different in *N. notopterus*. In the liver of *C. marulius*, trace elements concentration revealed the given descending manner: Al>S>Fe>Si>Zn>Cu>Cr=Mn>Sr (Table 6) while the order observed in *B. bagarius* was Al>S>Fe>Si>Zn>Cu>Mn>Cr>Sr (Table 5), in *N. notopterus* the order of finding the trace elements in the liver is S>Fe>Si>Al>Zn>Sr>Cu>Mn>Cr (Table 7) and in *M. armatus* order was seen as Al>S>Fe>Si>Zn>Mn>Cu>Cr>Sr (Table 8).

The most important and fundamental component of the hemoglobin protein in animals is iron. Fish have higher iron contents in their liver and kidneys. Fe concentrations in freshwater fish were highest in the liver and lowest in the muscles according to [55] research. According to our findings, the kidney and liver had the highest Fe concentration, while muscles had the lowest concentration. In this study, sulphur was found to be the element with the highest concentration in the liver of *N. notopterus*, while Fe accumulation was shown to be significantly higher after sulphur. In liver, different trace elements were found in the given manner: S>Fe>K>Mg>Si>Al>Zn>Sr>Ti>Cu>Mn>Cr>Ba (Table 7).

Compared to the muscles, the liver and kidneys are the organs that accumulate Al the most. As cells treated with Al showed a decrease in their capability, indicating Al impeded DNA repair in Al treated cells, it has been shown that Al generated DNA damage in fish is dosage dependent [56]. All four fish species have significantly greater levels of Al buildup in their kidneys and liver. After ingestion, trace elements are carried through the bloodstream to the liver, a storage organ, where they can be transformed or bio-accumulated in different fish organs [57]. This liver's critical importance for fish metabolism and internal physiological functions is demonstrated by an investigation of freshwater species [58]. It was shown that the liver and kidneys often have higher metal concentrations than muscles. The muscles are the organs that acquire heavy metals the least [59].

According to the research, muscles were the key organs of metal concentration, followed by the kidney and liver. The succession of trace elements in the muscles of *C. marulius* was also noted as: S>Si>Fe>Al>Zn>Cu>Cr>=Sr>Mn (Table 6), while in *B. bagarius* order observed was as S>Al>Si>Fe>Zn>Cu>Cr>Mn>Sr (Table 5), In *N. notopterus*, manner of the trace elements was recorded as S>Al>Si>Fe>Zn>Sr>Cu>Mn>Cr (Table 7). Whereas in muscles of *M. armatus* observed order of accumulation of toxic trace elements was seen as: S>Al>Si>Fe>Zn>Mn>Cu>Cr>Sr (Table 8). This study found the buildup of toxic elements in certain tissues of fish in the given order: kidney, liver, skin, and muscles, as demonstrated by Rajkowska and M. Protasowicki [60] in tissues of species from Polish lakes.

The results of this study are in line with those of earlier investigations, which discovered that livers of freshwater fishes acquire Cu at a higher rate than other tissues [61, 62]. These findings are consistent with our results, which showed that the kidney and liver of *M. armatus* had the greatest and lowest Cu concentrations, respectively, followed by the kidney. Copper can also be found in *N. notopterus*, and it shares the same symmetry. The homeostatic system and the fish body's capacity to regulate itself can be damaged by higher quantities of Cu that can accumulate in kidney and liver tissue [63]. Contrary to the results of this investigation, Danabas and Ural [64] discovered that the muscle and gills of *Capeo tatrutta* had larger Cu contents than the liver. Through the bile duct and fish kidneys, extra Cu is removed from the liver tissue [62]. Cu is dissolved in the water when fish comes into direct contact with it, and it diffuses through their respiratory skin surface. Avenant-Oldewage and Marx [65] found that the amount of Cu buildup in muscles is negligible even when fish are present in an aquatic environment that is severely contaminated with metals. The amount of Cu found in freshwater fish organs serves as a sign of Cu prevalence in the environment. The toxicity of Cu in many fish species depends on a variety of factors, including the presence of organic waste, the water hardness and the fish's early growth stages [21].

In this study, the kidney and muscle of *N. notopterus* had the greatest Cr concentrations in contrast to its liver. Vaseem and Banerjee [66] discovered, in contrast to our study's findings, that in *L. rohita*, an Indian fish collected in the Gangs River, Cr is mostly accumulated in the kidneys and gills. The kidney, liver, and then muscles of *M. armatus* were found to have the highest concentrations of Cr. The effluents from tanneries, which are highly salt-laden with Cr and may be concentrated in freshwater fish, may be a significant problem, according to past studies. The liver and kidneys can absorb Cr more quickly than the skin and muscles. To reduce the amount of Cr in the species, fish liver detoxifies Cr, which is then removed from the its elementary tract [65].

All living things require the mineral zinc. Studies have shown that Zn can build up in fatty tissues more than other tissues and may affect the physiological capacity of fishes to spawn [67]. In this investigation, some freshwater fish's kidneys deposited more zinc than their muscle tissues. After analysis, Thompson et al. [67] collected fish from Lake Awassa in Ethiopia and concluded that the mean concentration of Zn in muscle tissues was lower than the corresponding concentration in the kidney and liver, which was considerably lower than the average Zn quantities present in the freshwater fish muscles of the present research.

Typically, S is produced from factories and refineries, where it leads to structural defects in freshwater fish larvae. The muscles of all four species of fish contained the most of this chemical, according to our analysis. The most dangerous substance detected in the kidneys of *C. marulius*, *B. bagarius*, *N. notopterus*, and *M. armatus* was S followed by Fe. Fish liver and kidneys contained the highest amounts of Fe.

Tekin Özan and Aktan [68] found that the liver and muscles of freshwater fish had the greatest and lowest Fe contents, respectively. The kidney and liver had the highest Fe concentrations according to our results, and muscles across all the four species had the lowest concentrations.

Due to the serious threats they pose to human life, Mn, Sr, and Si are also priority trace elements with severe public health consequences. All of these metals are believed to be exceedingly dangerous, even at low quantities, and can damage a variety of body systems.

Due to its production of the metallothionein enzyme, which can bond with potentially dangerous trace metals, the liver may be a target organ for the accumulation of trace elements while the muscle tissues of freshwater fish are shielded by their skin from the hazardous substances in the environment. Little metal is transmitted from the fish's other body parts to its muscles since they cannot be pierced by foreign metals and cannot detoxify heavy metals like the liver does [69]. The muscles are the most crucial freshwater fish edible element for humans because other organs like the liver, and kidneys are eliminated before consuming fish [20]. The concentration of hazardous trace elements in fish muscles is much less than in other corresponding tissues.

V. CONCLUSION

In the current study, the concentrations of trace elements (Al, S, Fe, Si, Zn, Cu, Cr, Sr, Mn) and five macro-elements (C, Na, K, P, Mg) were measured in the kidney, muscles and liver of four freshwater fish species, *B. bagarius*, *C. marulius*, *N. notopterus*, and *M. armatus* from the Head Trimmu reservoir on the Chenab River. The quantities of macro-elements in fish muscles were generally higher than in the liver and kidney. The muscle of *N. notopterus* had the greatest mean Ca and P concentrations while the muscle of *C. marulius* also had the greatest mean K and Na concentrations. The kidney of *N. notopterus* had the highest mean content of Mg. The concentration of toxic trace elements was higher in fish liver and kidney than in muscles. The most prevalent element among four species was Al detected in the kidney of *M. armatus*. Sulphur was shown to be the second most prevalent element in the muscle of *C. marulius.* While the largest concentrations of Si, Zn, Cu, and Mn were found in the kidney of *M. armatus*, Fe was found to be the most concentrated element in the kidney of *N. notopterus*. While the liver of *N. notopterus* had the highest concentration of Sr and the kidney had the highest concentration of Cr. The mean concentrations of Cr, Cu, Fe, Si and Zn in tissue of all four freshwater species were significantly higher due to wastewater emission from industries and domestic use. Among toxic trace elements, Al, Mn, S and Sr had a significant relation between fish tissue and fish species (Two-way ANOVA, $p<0.05$). Maximum concentration of trace elements in muscle did not exceed permissible limits. Both THQ and TTHQ (HI) values were lower than 1, indicating that non-carcinogenic health issues are not seen for consumers. Thus, the consumption of *B. bagarius, C. marulius*, *M. armatus* and *N. notopterus* from the Head Trimmu reservoir is theoretically safe for humans.

FUTURE RECOMMENDATIONS

The current work would be helpful for

- Comparative assessment of trace elements concentration and their effects on fish tissues from selected sites of river Chenab, Pakistan.
- For the purpose of assessing health concerns associated with fish eating, it is advised to regularly evaluate metal accumulation in freshwater fishes of the Chenab River.
- The section of the River Chenab and its tributaries that passes through an industrial or urban region should be regularly monitored for the quality of its fish, sediment, and water, and prompt management actions

should be made. Communities in close proximity are aware of the detrimental effects that fish and water pollution have on people and the health of ecosystems.

COMPETING INTERESTS

The authors declare that they have no competing interests or personal relations that could have proven to affect the work reported in this paper.

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