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Effect of Varying Dietary Protein Levels on the Concentration of Some Essential and Non-essential Metals in *Catla catla*

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ABSTRACT

To investigate the concentration of macroelements (Na and K), microelements (Fe and Zn), and toxic heavy metals (Cd and Pb), fish samples of *Catla catla* were collected from the various treatments fed artificial fish feed containing 15, 20, 25, and 30% crude protein in T1, T2, T3, and T4, respectively, under a polyculture system for 180 days. Elemental concentrations in the fish samples were determined using a Flame Atomic Absorption Spectrophotometer. Mean Na, K, Fe and Zn concentrations (μgg^{-1}) in wet body weight of C. *catla* were ranged 379.44 -396.78, 237.89 - 262.71, 58.58 - 95.05 and 14.47 - 16.55 μgg^{-1} in different treatments. Cd and Pb were found below detectable limits in the body of *C. catla* in all studied treatments. Analyzed metal concentrations were found to be lower than the permissible limits proposed by different international organizations (FAO and WHO). For all studied treatments, the metal concentrations increased in the order: Cd=Pb<Zn<Fe<K<Na in the present work. An elevated trend was noticed among the essential metals, and a decreased trend among the nonessential metals was observed in the fish. Potassium concentrations in the whole body of *C. catla* showed differences ($p < 0.05$); however, iron, sodium, and zinc concentrations showed no significant difference among the studied treatments. Moreover, the size of the fish was found to be significantly correlated with Na and K, while remaining constant with Fe and Zn concentrations. Overall, various protein levels in fish feed do not have considerable effects on *C. catla* when reared in a polyculture system.

Keywords: *Catla catla*, Artificial fish feed, Dietary protein, Pond culture, Metal concentrations, Fish size.

1. INTRODUCTION

Fish might be an important dietary source of protein (Naeem et al., 2024; Ishtiaq et al., 2026) and essential mineral elements, which are important constituents of the human diet (Lilly et al., 2017; Kousar et al., 2021a,b). Several important minerals are generally present in fish flesh that include calcium, potassium, sodium, magnesium, iron, copper and zinc (Khalid et al., 2019; Riaz and Naeem, 2023), and may supply a variety of essential minerals to the human body upon consumption. Thus, fish has a significant potential to enhance intake of essential minerals, which is considered food based sustainable strategy (Kawarazuka and Bene, 2011). Several essential micronutrients, in trace concentrations, help to maintain the metabolism of the human body, but at elevated concentrations these metals become poisonous to humans (Edwards, 2010). Being the top of aquatic food chain, fish may accumulate various metals in

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large concentrations from their ecosystem (Mansour and Sidky, 2002; Naeem et al., 2012). Hence, fish species have been extensively used in pollution monitoring and the health of aquatic environment as studies have indicated that fish accumulate heavy metals to concentrations many times higher than those present either in sediments and/or water (Bat et al., 2012).

Aquatic organisms accumulate heavy metals from their food, bed sediments and water column, mostly coming from agricultural and industrial waste or human activities, either by absorption or ingestion. Other sources may include air depositions of aerosol dust, soil erosion and runoff (Goodwin et al., 2003). In farming systems, fishes are usually fed on artificial diets; and are typically supplemented with various metals including arsenic, cobalt, copper, iron, magnesium, manganese, selenium and zinc to fulfill their mineral requirements (CIESM, 2007). Alternatively, various ingredients of fish the artificial diet may themselves be the source of these metals. On the other hand, potential water sources used in fish ponds may also contribute high concentrations of these metals (El-Nahhal, 2006). Fish from the farming systems may absorb the pollutants from the water and feed, leading to accumulation in different body parts (McCarthy and Shugart, 1990) and thus may also pose toxicological effects to the consumers (Ali and Abdel-Satar, 2005).

Sodium (Na) is considered good for muscle functioning (Alas et al., 2014). It is also important to control osmotic pressure and acid-base balance. Like sodium, potassium (K) is also required for muscle contractions, transmission of nerve impulse and metabolic breakdown of glucose (Palmer, 2014). Iron (Fe) is considered one of the most significant micronutrients that is required for normal functioning of the tissues and body organs of animals including fish. However, excessive concentration of Fe may accumulate in liver, heart, pancreas and body tissues (Kundu et al., 2017). Zinc (Zn) serves as an important constituent of many enzymes as a cofactor and is responsible for numerous vital biological functions (Mogobe et al., 2015). But it is likely to have a toxic risk to health for the population of children, if taken in excess amunt. Ocular and dental disorders have been reported in populations exposed to elevated levels of certain metals; however, the potential involvement of essential elements such as zinc and iron requires further investigation (Milend Mbeh et al., 2019). Cadmium (Cd) is a non-essential, highly toxic and ecotoxic metal. The occupational levels of cadmium exposure prove to be a risk factor for testicular degeneration and chronic lung disease (Li et al., 2005). Lead (Pb) poisoning in humans causes severe dysfunction in the kidney, reproductive system, liver, brain and central nervous system (Martínez-Quintana and Penagos-Corzo, 2012).

Excessive concentrations of heavy metals impair ecosystems as some of them can cause potential bioaccumulation in living organisms (Majed et al., 2019). The bioaccumulation of heavy metals in edible fish species may be considered as a warning for the negative impacts of fish consumption on human health (Maurya et al., 2019; Mahida et al., 2026).

Despite the availability of numerous studies on the nutritional composition and toxicity of cultured fish, limited information exists regarding the influence of dietary protein levels on the accumulation of essential and potentially toxic elements in *Catla catla* reared under polyculture farming conditions. Dietary protein plays an important role in fish growth, metabolism, and nutrient utilization and may influence the uptake and accumulation of mineral elements in fish tissues. While Na, K, Fe, and Zn are essential for normal physiological functions, Pb and Cd are toxic elements that may pose risks to fish health and human consumers when accumulated at elevated concentrations. However, the effects of different dietary protein levels on the concentrations of these elements in farmed *C. catla* remain insufficiently documented. Therefore, this study aims to investigate the effects of 15%, 20%, 25%, and 30% dietary protein levels on the concentrations of Na, K, Fe, Zn, Pb, and Cd in *C. catla* reared under a polyculture farming system. The study will provide information relevant to feed formulation, fish health, aquaculture management, and the food-safety assessment of farmed *C. catla*.

2. MATERIALS AND METHODS

2.1. Feeding Trial and Sample Collection

Catla catla were reared in earthen ponds under a polyculture system by feeding various levels of crude protein (CP) diets, 15%, 20%, 25% and 30% CP, in different treatments, T1, T2, T3 and T4, respectively, for 180 days (Ishtiaq, 2019). At the end of the feeding trial, 30 fish samples from each of four treatments, and a total of 120 fish samples were collected by using drag net, and analyzed to study the effect of various levels of dietary crude protein (CP) on the metal concentrations. Furthermore, effect of body size on the metal concentrations was also studied from different treatments. Total length and wet body weight of each fish sample were recorded to study the effect of body size on the metal concentrations was also studied from different treatments.

2.1.1. Metals Analysis

All of the collected fish samples were dried, dry body weight was recorded, and ground by using electronic grinding machine to make homogenized powder. A 1g sub-sample from each sample powder was taken and ash was prepared using muffle furnace (RJM-1.8-10A). The

resulting ash was digested with 10 mL of 70% nitric acid (HNO_3) on a hot plate at 82–100°C. The digest was heated until dryness and subsequently diluted to a final volume of 25 mL with deionized water following Naeem et al. (2011a) and Khalid et al. (2019). The prepared solutions were aspired into Flame Atomic absorption spectrophotometer (Thermo Scientific: iCE 3000 Series) at Food Analysis Lab, Institute of Food Science & Nutrition, Bahauddin Zakariya University, Multan, Pakistan, for quantification of Cadmium (Cd), Iron (Fe), Potassium (K), Sodium (Na), Lead (Pb) and Zinc (Zn) concentration in the fish samples of *Catla catla*, which were fed various level of dietary protein in fish feed. Blank solutions and calibration standards were analyzed using the same procedure as that applied to the fish samples. Instrument calibration standards were prepared by serial dilution of certified 1000 mg/L (1000 ppm) stock standard solutions supplied by Merck, Germany. A known 1000 mg/L stock solutions of the respective metals were prepared from their analytical-grade salts. Instrument calibration was verified and, recalibrated after every 10 samples during the analytical run.

2.1.2. Statistical Analyses

One-way analysis of variance (ANOVA) was also determined to evaluate the effects of various crude protein levels on the metal concentrations in *Catla catla*, collected from different treatments. Duncan's Multiple Range Test was used to determine differences among various studied treatments, at $p<0.05$ level of significance.

To evaluate the relationship of body size with different analyzed metal concentrations in the samples of *C. catla*, regression analyses were performed by using the equation:

$$Y = a + b\,X$$

Where, Y= metal concentration ($\mu g g^{-1}$); X = Fish length (cm) or Weight (g); a= constant; b= slope

Multiple regression analysis (MRA) was also carried out to determine the inter-relationships of fish size and metal concentration. Correlation coefficients were considered significant at $p<0.001$, $p<0.01$ and $p<0.05$, for regression analyses. Log transformed data was statistically analyzed to explain allomeric pattern of metal concentration in the fish by using *b*-value. Statistical analyses were applied by using MiniTab and SPSS (version 23.0).

3. RESULTS

3.1. Metal Concentrations in *Catla catla*

The data obtained for metal concentration ($\mu g g^{-1}$) of Na, K, Fe, Zn, Cd and Pb in wet and dry body weight of analyzed fish, *Catla catla*, reared under a polyculture system, are presented in

Table 1. Descriptive for element concentration (µgg^{-1}) in wet (WW) and dry body weight (DW) of Catla catla, reared under polyculture system.

Elements	***Wet Weight/ Dry Weight***	***Treatment 1 (15% CP)***		***Treatment 2 (20% CP)***		***Treatment 3 (25% CP)***		***Treatment 4 (30% CP)***		***p value***
		Range	***Mean± S.E.***	***Range***	***Mean± S.E.***	***Range***	***Mean± S.E.***	***Range***	***Mean± S.E.***	
Na	WW	178.51-759.68	396.78±25.76	214.78-643.63	400.45±20.76	232.28-771.45	394.46±27.28	237.40-676.27	379.44±20.14	0.928
	DW	974.50-3026.75	1696.23±102.45	961.75-2870.00	1747.66±86.51	896.75-2937.50	1566.13±106.62	975.00-2761.25	1521.70±78.83	0.286
K	WW	191.76-297.03	247.94±5.52 ^{a,b}	179.99-313.43	238.52±5.48^{b}	205.94-305.78	251.94±4.72 ^{a,b}	214.91-329.90	262.71±5.52^{a}	0.017
	DW	826.25-1242.50	1066.26±18.91	886.00-1225.25	1039.97±18.01	875.00-1188.00	999.94±17.03	877.50-1230.50	1054.92±19.46	0.064
Fe	WW	0.41-423.59	95.05±19.20	0.00-464.64	86.44±21.54	0.00-272.99	72.57±15.45	0.00-224.26	58.58±10.94	0.464
	DW	1.61-1746.89	409.53±81.93	0.00-1981.29	374.23±93.20	0.00-1073.91	285.11±60.45	0.00-900.88	236.73±44.64	0.313
Zn	WW	1.94-32.37	16.55±1.48	1.70-29.78	15.77±1.49	0.00-32.19	14.59±1.97	0.41-34.86	14.83±1.32	0.808
	DW	7.71-133.55	71.88±6.49	6.77-122.50	67.98±5.86	0.00-129.16	57.99±7.74	1.70-139.04	59.92±5.45	0.377
Cd	WW	ND	ND	ND	ND	ND	ND	ND	ND	--
	DW	ND	ND	ND	ND	ND	ND	ND	ND	--
Pb	WW	ND	ND	ND	ND	ND	ND	ND	ND	--
	DW	ND	ND	ND	ND	ND	ND	ND	ND	--

Note: S.E. = Standard Error; ND= Not Detected (Below detection limit); Mean values sharing the same superscript are not significantly different (p>0.05).

table 1 and figure 1. The concentrations of Na, K, Fe and Zn in the whole body of *Catla catla* were found to be 392.78±11.72, 250.28±2.74, 78.16±8.61 and 15.43±0.78 µgg^{-1}, respectively, for overall combined data (Fig 2). Results of analysis of variance (ANOVA) indicated no significant difference (p>0.05) in Fe, Na and Zn concentrations in both wet body weight (WW) and dry body weight (DW) of *Catla catla* among different treatments. However, potassium concentration (µgg^{-1}) was found significantly higher (p<0.05) in T4, in which fish were fed artificial feed containing 30% crude protein.

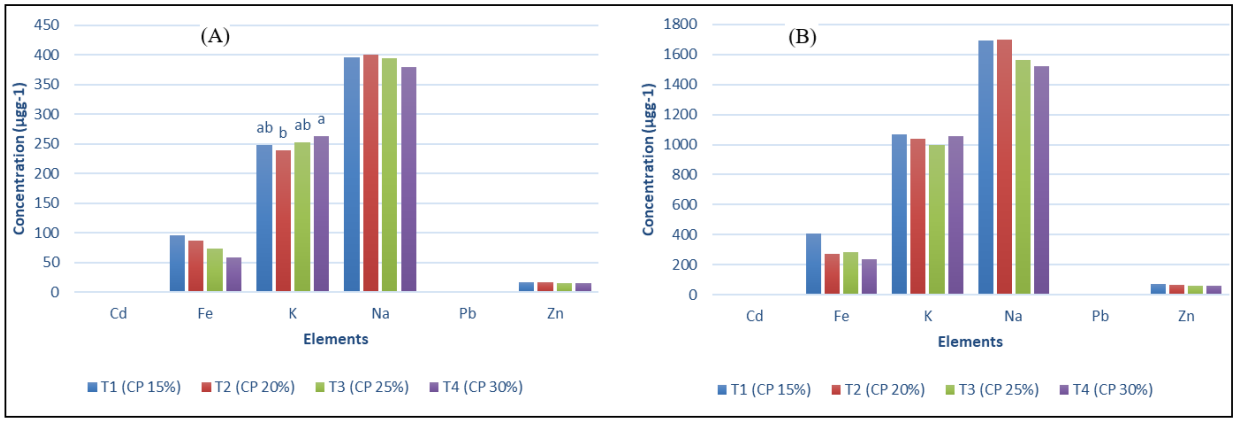


Figure 1. Element concentration (µgg^{-1}) in wet (A) and dry body weight (B) of Catla catla, fed upon a diet containing 15%, 20%, 25% and 30% CP, reared under polyculture system.

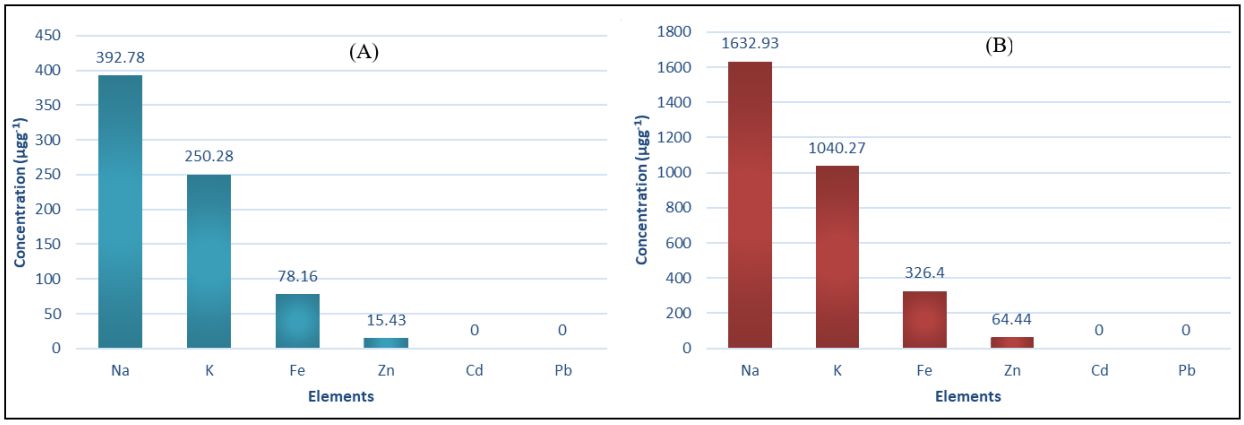


Figure 2. Overall combined element concentration (µgg^{-1}) in wet (A) and dry body weight (B) of Catla catla.

Cadmium and lead levels were found below the detection limit in all studied treatments, fed with 15%, 20%, 25% and 30% crude protein (CP) in T1, T2, T3 and T4, respectively. Iron (Fe) was also found below the detection limit in some samples of *C. catla,* collected from T2, T3 and T4. However, maximum concentration of Fe (wet weight of fish body) was found 464.64 µgg^{-1} in the body of *C. catla* fed upon 20% CP (T2) in artificial fish feed. Potassium (K) concentration was

ranged 179.99−329.90 µgg^{-1} in wet body weight and 826.25−1242.50 µgg^{-1} in dry body weight of *C. catla*. Minimum sodium concentration was noted 178.51µgg^{-1} in T1 while the highest value (771.45 µgg^{-1}) was found in T3 in the wet body weight of *C. catla*. Mean concentration of zinc metal in the analyzed samples of *Catla catla* was ranged 14.59−16.55 µgg^{-1} among different treatments.

3.2. Correlation between Fish Size (Weight and Length) and Metal Concentration

Log transformed wet weight (W) was found highly significantly (p<0.001) correlated with log-transformed potassium and sodium concentration across in all treatments, except for log transformed Na concentration in T3 which was least significantly (p<0.05) correlated the with wet body weight of the *C. catla*. Concentrations of Na and K exhibited positive allometry (b>1.0) with an increasing body weight of *C. catla*, reared under polyculture system, across all studies treatments, except for Na concentration in T3 which showed isometry (b value not significantly different from 1.0). However, Fe and Zn concentrations were found insignificantly (p>0.05) correlated with the wet weight of *C. catla*, reared under a polyculture system, in all studies treatments (Table 2).

Table 2. Regression parameters of wet body weight (WW, g) versus body burden element (µgg^{-1}) for Catla catla, reared under polyculture system, in different treatments.

Equation	***Treatment***	***r***	***a***	***b***	***S. E. (b)***	***t value when b=1***
Log Na =a + b Log WW	T1	0.660***	-0.165	1.949	0.419	-0.438
	T2	0.633***	1.631	1.330	0.307	-1.927
	T3	0.411*	2.422	1.050	0.440	-1.223
	T4	0.609***	1.199	1.462	0.360	-1.316
Log K =a + b Log WW	T1	0.837***	1.711	1.236	0.152	-5.343
	T2	0.927***	1.190	1.409	0.108	-7.850
	T3	0.900***	1.540	1.291	0.118	-7.184
	T4	0.863***	1.647	1.261	0.140	-5.882
Log Fe =a + b Log WW	T1	0.325^{ns}	-6.841	3.919	2.157	3.455
	T2	0.107^{ns}	11.112	-2.551	4.479	-2.774
	T3	0.154^{ns}	-7.248	3.788	4.604	3.571
	T4	0.025^{ns}	4.437	-0.136	1.045	-1.093
Log Zn =a + b Log WW	T1	0.112^{ns}	2.581	0.503	0.841	-0.686
	T2	0.324^{ns}	0.105	0.251	1.302	-0.517
	T3	0.155^{ns}	13.958	-3.533	4.259	-3.768
	T4	0.025^{ns}	4.437	-0.136	1.045	-1.093

Note: ns = non-significant, * = p<0.05, *** = p <0.001.

Insignificant (p>0.05) correlation was noted between total length (TL) and Fe concentration in log transformed data for all treatments. Correlation was found highly significant (p<0.001) between log TL and log K in all treatments. Potassium concentration showed positive allometry (b>3) with an increase in TL of farmed *C. catla.* On the other hand, log TL represented highly significant (p<0.001) relationships in T1 and T2, in which *C. catla* were fed 15% and 20% CP, respectively. Log TL showed least significant correlation at p<0.05 with log Na in T3, in which *C. catla* were fed 25% CP, while significant correlation at p<0.01 in T4, in which the fish were fed 30% CP. Positive allometric pattern (b>3) was observed in Na concentration with TL for all studied treatments. Log Zn concentration showed least significant (p>0.05) correlation with log TL in T2, with positive allometric pattern (Table 3).

Table 3. Regression parameters of total length (TL, cm) versus body burden element (µgg^{-1}) for Catla catla, reared under polyculture system, in different treatments.

Equation	***Treatment***	***r***	***a***	***b***	***S. E. (b)***	***t value when b=3***
Log Na =a + b Log TL	T1	0.701***	-4.089	6.020	1.159	3.432
	T2	0.619***	-0.718	3.902	0.936	0.697
	T3	0.380*	0.322	3.238	1.489	1.223
	T4	0.502**	-0.916	4.006	1.306	1.709
Log K =a + b Log TL	T1	0.814***	-0.270	3.498	0.472	-2.858
	T2	0.914***	-1.362	4.174	0.350	-4.397
	T3	0.881***	-1.415	4.213	0.427	-2.813
	T4	0.792***	-0.811	3.850	0.561	-1.498
Log Fe =a + b Log TL	T1	0.322^{ns}	-13.460	11.304	6.286	10.827
	T2	0.056^{ns}	10.040	-3.972	13.504	-4.194
	T3	0.117^{ns}	-11.555	9.648	15.425	9.454
	T4	0.066^{ns}	-4.416	5.253	15.008	5.053
Log Zn =a + b Log TL	T1	0.114^{ns}	1.669	1.490	2.449	0.265
	T2	0.380*	-3.259	4.581	2.108	3.158
	T3	0.128^{ns}	19.207	-9.765	14.252	-9.975
	T4	0.020^{ns}	4.638	-0.375	3.478	-1.238

Note: ns = non-significant, * = p <0.05, ** = p <0.01, *** = p <0.001.

3.3. Multiple Regression Analyses

Different parameters for multiple regression analyses among total body burden of metals, total length (TL, cm) and wet body weight (WW, g) for *C. catla*, reared under polyculture system, in different treatments are represented in table 4. Iron was observed least significant (p < 0.05) in T1, whereas significant (p < 0.01) in T3 with WW and TL. Relationships were found insignificantly (p > 0.05) correlated in T2 and T4 for *C. catla*. Potassium showed highly significant correlation

($p < 0.001$) in all studied treatments. Sodium was remained significantly correlation with WW and TL in T1, T3 and T4 at $p < 0.01$, $p < 0.05$ and $p < 0.001$, respectively, while showed no significant correlation in T2 for *Catla catla*. Concentration of zinc metal was found significant ($p<0.001$; r = 0.608) only in T2 with WW and TL of *C. catla*.

Table 4. Multiple regression analyses of wet body weight (WW, g) and total length (TL, cm) with total body burden of metal for Catla catla, reared under polyculture system, in different treatments.

Equation	***Treat-ments***	***r***	***a***	***b_1 ± S.E***	***b_2 ± S.E***	***r^2***	***VIF***
Na = a + b_1 WW + b_2 TL	T1	0.692**	-3617600	-1648.6±828.9	135049±47944	0.479	3.248
	T2	0.214 ns	236	0.1705±0.7931	0.74±47.25	0.046	1.273
	T3	0.402*	713308	840.3±711.7	-27627±52369	0.162	1.674
	T4	0.669***	1736949	1405.9±410.6	-66026±29861	0.448	3.024
K = a + b_1 WW + b_2 TL	T1	0.840***	183340	463.3±204.5	-9060±11830	0.705	6.236
	T2	0.908***	-145693	288.1±147.3	2794±8778	0.824	10.839
	T3	0.881***	-111205	298.1±108.6	1740±7992	0.776	8.397
	T4	0.866***	339457	527.1±119.6	-14313±8698	0.750	7.464
Fe = a + b_1 WW + b_2 TL	T1	0.396*	- 390061	142.2±709.1	9253±41012	0.157	1.656
	T2	0.277 ns	-1218600	-614.1±684.6	45774±40793	0.077	1.384
	T3	0.512**	985415	821.6±355.6	-41115±26168	0.262	2.049
	T4	0.114 ns	-245608	-138.1±238.0	10483±17314	0.013	1.129
Zn = a + b_1 WW + b_2 TL	T1	0.311 ns	-99053	-53.63±59.26	3980±3428	0.097	1.452
	T2	0.608***	-182465	-84.08±44.00	6752±2622	0.370	2.553
	T3	0.176 ns	-45876	-8.33±51.48	1649±3787	0.031	1.214
	T4	0.221 ns	-58391	-30.86±29.08	2466±2115	0.049	1.284

Note: ns = non-significant, * = p <0.05, ** = p <0.01, *** = p <0.001.

3.4. Inter-elemental Relationships

To find the inter-elemental relationship, regression analyses between analyzed metal concentrations (wet body weight) of *C. catla*, reared under polyculture system were performed and correlation coefficients are given in table 5. Various relationships between metal concentrations *i.e.* K-Fe, Na-Fe, Zn-Fe, Na-K, Zn-K, Zn-Na were evaluated by using regression analyses. Results showed that only concentration of zinc metal was significantly correlated with iron in *C. catla.*

Table 5. Inter-elemental correlation matrix of metals in wet body weight of Catla catla, reared under polyculture system, for overall data (n=120).

Metals	***Na***	***K***	***Fe***	***Zn***
Na	1			
K	0.158 ns	1		
Fe	0.023 ns	0.006 ns	1	
Zn	0.050 ns	0.018 ns	0.380*	1

Note: ns = non-significant, * = p <0.05.

4. DISCUSSION

4.1. Metal Concentrations

The study aimed to evaluate the effect of dietary protein levels on some metal concentrations in *Calta catla*. Dietary protein concentration can significantly impact physiological processes in fish, including nutrient absorption, metal accumulation, and detoxification mechanisms (Abdel-Tawwab et al. 2010; Jiang et al., 2025). Therefore, varying dietary protein levels allowed us to investigate how nutritional status affects the distribution of metals in fish tissues. Results of the present study describe that Potassium concentration was found significantly different (p<0.05) among various treatments in *C. catla*, indicating an increase in the concentration of potassium content with an increase in dietary protein in fish feed. Whereas, no significant difference exists among different treatments for Na, Fe and Zn concentration in the body of *C. catla*. Hence concentrations of these essential metals in *C. catla* remained constant when fed upon different levels of protein (15, 20, 25 and 30% CP) in diet. Moreover, metal concentrations on a dry-weight basis remain higher as also expressed in this study (Table 1) because drying removes tissue moisture while the metals remain in the residual dry matter, thereby concentrating the analytes per unit mass. Thus, dry- and wet-weight values are directly related through the moisture content of the fish tissue (Naeem et al., 2011a).

Iqbal et al. (2019) have also examined the effect of graded levels of dietary protein (15, 20, 25% CP) on elemental concentration of hybrid (*Catla catla* ♂ ***x*** *Labeo rohita* ♀), and reported iron 84.89±6.43 to 132.62±5.91, potassium 1610.79±114.04 to 2049.44±93.43, sodium 842.12±78.61 to 1060.52±68.42, zinc 35.88±2.36 to 46.78±2.69, cadmium 0.102±0.044 to 0.105±0.039 and lead concentration 0.36±0.04 to 0.38±0.03 $\mu g g^{-1}$, with an increasing trend of essential elements with rise in protein level in diet for hybrid fish. Kousar et al. (2021a) have also examined the effect of graded levels of dietary protein (15, 20, 25% CP) on elemental concentration of Genetically Improved Farmed Tilapia (GIFT), and reported iron 125.26 ± 80.58 to 198.81 ± 60.97, zinc 35.26 ± 10.14 to 44.70 ± 7.64, cadmium 0.10 ± 0.02 to 0.11 ± 0.08 and lead concentration 0.30 ± 0.05 to 0.40 ± 0.10 $\mu g g^{-1}$, with an increasing trend of essential elements with rise in protein level in diet for GIFT. The variations might be due to differences in size or the fact that the accumulation of metals can be species-specific. As, dynamics of heavy metal metabolism in the fish species and their respective correlation with the water quality parameters

may not be the same for all the heavy metals and even for different fish species (Majed et al., 2019).

Comparison of concentration in whole fish of *Catla catla* with various studies is provided in table 6, while permissible limits for heavy metals recommended by various organizations are given in table 7. For all studied treatments, the metal concentrations increased in the order: Cd=Pb<Zn<Fe<K<Na in the present work. A declined pattern was noticed among non-essential metals, while an increasing trend was observed among essential metals in the body of fish.

By comparing with other studies (Table 6), it can be inferred that Sodium (Na) content in the present study was found to be lower than Tariq et al. (1993), Salam et al. (1998) and Naeem et al. (2011a). Potassium (K) concentration, in the present study, is in conformity with the outcomes of Naeem et al. (2010; 2011a) from farming system. Conversely, Tariq et al. (1993) reported higher concentration of K in wild *C. catla*, suggesting that wild captured fish may contain more potassium content than farmed fish, possibly due to greater variety of food available in natural environment. Another factor that may be involved in lower concentration of this essential mineral, is the feeding habits and food competition under a polyculture system. Additionally, seasonal and habitat-related variations in K concentration have also been reported in Pakistani fish species (Gull et al., 2024).

Results showed that the mean concentrations of iron (Fe) in the analyzed fish samples of *Catla catla* ranged from 58.58 ± 10.94 to 95.05 ± 19.20 μgg^{-1} in different treatments, while it was 78.16 ± 8.61 μgg^{-1} for overall data. Fe content was found lower than the permissible limit of 80 μgg^{-1} (Kundu et al., 2017), in the samples of *Catla catla* collected from T2 (20% CP), T3 (25% CP), T4 (30% CP) and for overall data, however, it was higher in T1 (15% CP), which might be due to difference in feed formulation. Fe concentrations documented by Naeem et al. (2010; 2011a) is very close to the present study. The higher Fe content reported by Kundu et al. (2017) in the farmed fish body may be due to the feed, which was likely rich in iron.

In this investigation, Zn concentration remains below the permissible limits (Table 6), in all studies treatments, by feeding varying dietary protein levels. Furthermore, the results found very close to the study of Naeem et al. (2010). On the other hand, Tariq et al. (1993) have reported lower Zn content, representing that *Catla catla* from the farming system is a source of high quality of zinc, even cultured under polyculture system. Moreover, it also indicates that fish feed formulated in the study, provided adequate amount of zinc to the fish.

Table 6. Comparison of concentration in whole fish (μgg^{-1}, wet weight) among various studies.

Fish	***Na***	***K***	***Fe***	***Zn***	***Cd***	***Pb***	***Reference***
Catla catla (Site-1)	829 ±242	2714 ±681	2.193 ±0.547	2.003 ±0.492	4.701 ±1.026	2.973 ±0.714	Tariq et al. (1993)
Catla catla (Site-2)	74 ±18	2175 ±546	3.640 ±1.006	1.036 ±0.312	0.211 ±0.009	1.832 ±0.425	
Catla catla	537.0 ±107.0	2046.0 ±470.0	145.0 ±44.0	23.0 ±3.2	ND	3.2 ±0.6	Salam et al. (1998)
Oncorhynchus mykiss	339.42 ±15.191	1470.07 ±38.893	38.57 ±1.758	28.37 ±1.132	0.33 ±0.039	1.63 ±0.174	Naeem et al. (2010)
Aristichthys nobilis	770.48 ±26.57	1631.94 ±63.62	61.64 ±6.21	15.49 ±1.41	0.40 ±0.02	2.25 ±0.19	Naeem et al. (2011a)
Mystus bleekeri	--	--	--	16.96 ±1.13	6.27 ±0.88	--	Naeem et al. (2012)
Wallago attu	--	--	21.672 ±7.133	22.274 ±2.028	0.072 ±0.019	0.475 ±0.188	Yousaf et al. (2012)
Hybrid (*Catla catla*♂ x *Labeo rohita*♀)	1060.52 ±68.42	2049.44 ±93.43	132.62 ±5.62	46.78 ±2.69	0.105 ±0.03	0.38 ±0.03	Iqbal et al. (2019)
Ctenopharyngodon idella	904.04 ±904.04	1965.18 ±75.35	49.16 ±4.81	22.29 ±0.94	0.004 ±0.003	0.25 ±0.03	Khalid et al. (2019)
Notopterus chitala	255.51 ±32.45	1327.15 ±234.71	--	--	--	--	Zafar et al. (2022)
Glossogobius giuris	--	--	--	--	ND	0.05 ± 0.01	Agtong et al. (2023)
Cyprinus carpio	--	--	6.43 ±1.254	11.265 ±0.032	0.045 ±0.061	--	Almafrachi et al. (2024)
Wallago attu	204.14 ± 36.23	1230.00 ±144.46	15.71 ±7.08	9.56 ±4.39	0.0001 ±0.0003	2.47 ±0.87	Riaz and Naeem (2024)
Scomberoides commersonnianus	589.16 ±247.20	896.62 ±268.85	41.60 ±17.20	59.32 ±53.06	0.04 ±0.09	0.20 ±0.29	Azam and Naeem (2024)
Catla catla	208.79 ±83.70	528.74 ±120.89	10.41 ±2.95	10.85 ±4.67a	ND	ND	Rehman et al. (2026)
Catla catla	392.78 ±11.72	250.28 ±2.74	78.16 ±8.61	15.43 ±0.78	ND	ND	Present Study

Note: ND = Not Detected

Table 7. Permissible limits and standard levels in (μgg^{-1}, wet weight) for metals recommended by various organizations in fish as described in literature.

Organization/Country	***Zn***	***Cd***	***Pb***	***Source***
FAO	30	0.05	0.5	FAO (1983)*
FAO/WHO limits	40	0.5	0.5	FAO/WHO (1989)**
Saudi Arabia	-	0.5	2.0	SASO (1997)**
England	50	0.2	2.0	MAFF (2000)**
EU Limits	-	0.1	0.1	EU (2001)**
European Community	-	0.05	0.2	EC (2005)**
Turkish Guidelines	50	0.1	1	Dural et al. (2007)**

Note: *cited in Burger and Gochfeld (2010); ** cited in Elnabris *et al*. (2013).

Cadmium and lead were found below detectable limits in the body of *C. catla* in all studied treatments indicating safety for consumption in term of these heavy metals. Likewise, according to other studies, Cd was also not quantifiable in hatchery reared *Oreochromis niloticus* (Salam et al., 1996), wild *O. niloticus* (Naeem et al., 2011b) and *Glossogobius giuris* (Agtong et al., 2023). While, Chatta et al. (2017), Iqbal et al. (2019) and Khalid et al. (2019) have documented Cd and Pb concentrations (Table 6) within permissible limits in farmed carp fish species. These interpretations are in accordance with the outcomes of this research work. On contrary, Naeem et al. (2012) have recorded higher value of cadmium concentration (6.27±0.88 $\mu g g^{-1}$) in a wild captured catfish (*Mystus bleekeri*). Qadir and Malik (2011) and Rauf et al. (2009) also reported higher concentration of Cd in wild captured *Oreochromis niloticus* (3.120 $\mu g g^{-1}$) and *Catla catla* (2.58 $\mu g g^{-1}$), respectively (Table 6). This confirms that fish from the farming system is safer in term of Cd and Pb toxicity as compared to wild fish. This finding is further supported by another study by Suleman et al. (2025), reporting that water sources of Pakistan are impacted by toxic metals, affecting the consumable fishes but farmed fishes are less contaminated than the riverine fishes of Pakistan due to reduced exposure to industrial effluents.

4.2. Effect of Body Size of Fish on Metal Concentration

Besides investigating the effect of varying dietary levels on the elemental composition in *Catla catla* reared in polyculture system, effect of body size of fish on metal concentration was also assessed by using regression equation. Different scientists (Salam et al., 2002; Hussain et al., 2011; Naeem et al., 2011a,b, 2012; Yousaf et al., 2012; Fatima and Naeem, 2016) provided reliable estimates by using these predictive equations. Most of the studies reported a significant correlation between metal content and fish size. However, Hussain et al. (2011) have reported insignificant relationship of potassium concentration in *Catla catla* with its weight. Naeem et al. (2010) have described negative allometric pattern in Na and K with total length and weight of *O. mykiss*, while Naeem et al. (2011a) showed an increase in Na and K quantity with an escalation in weight of *A. nobilis.* However, the results of present study are in general agreement with the study of Salam et al. (1996), who reported no dependence of different metals on body size for *Oreochromis nilotica,* except for Na, K, Sn, and Pb which showed positive allometry. Thus, it is proposed that the concentration of Iron and Zn remained constant while potassium and sodium increased linearly with an increase in fish size, probably due to the reason that some metals accumulate at a higher rate while others at a lesser rate compared to their rate of excretion as fish grows in size.

4.3. Multiple Regression Analysis (MRA)

All the results were found in general agreement with Naeem et al. (2010) except for Fe in T2 and T4, Na in T2 and Zn in T1, T3 and T4, which remained constant with the growth of *Catla catla* in polyculture system. To find the multicollinearity, Variance Inflation Factor (*VIF*) was also calculated for MRA. All the predictive equations showed high multicollinearity (*VIF* > 10) with size of *C. catla* in all studied treatments, except for K in T2.

4.4. Inter-elemental Regression

Results show that the relationship only existed between Zn and Fe, while other metals remained constant with each other. Results were found in accordance with the study reported by Burger and Gochfeld (2010) and Naeem et al. (2012), who had stated significant correlation between some elements while insignificant in others. The results suggest that high or low concentration of a metal did not predict what the concentration of other metals would be. But still to evaluate the inter-elemental relationship is imperative as a pattern may emerge when other similar work is conducted with fish.

5. CONCLUSION

Overall, various protein levels in fish feed did not demonstrate considerable effects on the metal concentration in *Catla catla,* when reared in a polyculture system. Results showed that observed metal contents have lower concentration of mean values than the permissible limits set by FAO/WHO in the analyzed samples. Cd and Pb were not accumulated in *C. catla* which may be due to a pollution-free environment, supply of good quality feed or species specific. Although, essential mineral contents were found in lower, but non-availability of toxic and hazardous heavy metals in the *C. catla* from polyculture farming system, making it safe for human consumption. It can be concluded that *C. catla* has a good nutritional quality, when fed an artificial diet in farming system. Moreover, it can also be inferred by comparing the results with the reported studies that fish from the farming system is safer in term of heavy metal toxicity as compared to wild captured fish. In perspective, a comprehensive study on heavy metals in different fish species should be conducted over a long period in the farming system and different rivers of Pakistan.

6. SUPPLEMENTARY INFORMATION

This study is reported in accordance with S1 checklist completed, ‘‘The ARRIVE guidelines 2.0: author checklist’’, for reporting animal data (doi: 10.1371/journal.pbio.3000410). This paper does not contain any studies with human participants by any of the authors. The study did not involve endangered or protected species. Moreover, detail of the feeding trial is provided as supplementary information, available online: http://dx.doi.org/10.17582/journal.sja/2019/35.3.976.984

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8. CONFLICT OF INTEREST

The authors have no conflict of interest in conducting research and writing the manuscript.

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