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Comparative study among dried and homogenous digestion methods for essential element contents present in organs of two commercially important fish species collected from Indus River at Guddu Barrage

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Abstract

The aim of present study was to compare the efficiency of two methods for analyze of essential element contents in different parts of commercially important fish species Rohu (*Labeo rohita*) and Morakhi (*Cirrhinus mrigala*) collected from Guddu Barrage Sindh. The Essential elements Sodium (Na), Potassium (K), Calcium (Ca) and Magnesium (Mg) were evaluated by using Atomic Absorption Spectrophotometer AA-100. For comparative studies different sizes of fish species were sampled during year 2016 were shifted to the laboratories for performing dissection and digestions of respective fish organs i.e. gills, liver and muscle. Both homogenous and dried methods were employed for digestion of fish samples in order to evaluate the efficiency of both methods for best mineralization. Thus findings of present study were indicated that in Rohu fish samples, concentrations of essential elements Na, K, Ca and Mg were noted in the range of (0.35-2.9, 8.4-13.7, 8.0-50.0 & 3.5-33.6 $\mu\text{g.g}^{-1}$) and (1.0-11.76, 1.0-12.0, 0.5-9.8 & 5.3-35.4 $\mu\text{g.g}^{-1}$) for homogeneous and dried digestion methods respectively. While in case of Morakhi fish samples the concentration of respective metals were observed in range of (1.1-3.7, 0.3-13.0, 8.10-22.7 & 0.3-33.4 $\mu\text{g.g}^{-1}$) for homogeneous digestion and (1.0-10.4, 0.30-35.7, 1.1-10.0 & 3.0-37.5 $\mu\text{g.g}^{-1}$) for dried digestion method respectively. It has been observed that best mineralization results were achieved in the samples which were digested by Dried digestion method as compared to homogenous method due to different composition of tri-acids (HNO_3 , H_2SO_4 , HClO_4 in ratio of 1:4:8). While for the homogenous digestion method the composition of acids was (HNO_3 , HCl , H_2O_2 in ratio of 6:4:2).

So the dried digestion method was observed superior over the homogenous digestion method for evaluation for contents of essential elements in both fish organs of study area.

Keywords: AAS; essential minerals; fish species; homogenous digestion; Dry digestion

Introduction

Anthropogenic activities continuously increase the amount of elements in the environment, especially in aquatic ecosystem. Pollution of elements in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem ^[1]. Increase in population, urbanization, industrialization and agriculture practices have further aggravated the situation. As some elements cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals and thus, causing elemental pollution in water bodies. Therefore, elements can be bio-accumulated and bio-magnified via the food chain and finally assimilated by human consumers resulting in health risks ^[2]. As a consequence, fish are often used as indicators of heavy metals contamination in the aquatic ecosystem because they occupy high trophic levels and are important food source. Heavy metals are commonly found in natural waters and some are essential to living organisms, yet they may become highly toxic when present in high concentrations. These metals also gain access into ecosystem through anthropogenic source and get distributed in the water body, suspended solids and sediments during the course of their mobility. The rate of bioaccumulation of heavy metals in aquatic organisms depends on the ability of the organisms to digest the metals and the concentration of such metal in the river. Also it has to do with the concentration of the heavy metal in the surrounding soil sediments as well as the feeding habits of the organism ^[3].

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The bio-accumulation of heavy metals in living organisms and bio-magnifications describes the processes and pathways of pollutants from one trophic level to another. Many fish species which are widely used as bio-indicators of certain heavy metals contamination in aqua culture. The acidic conditions of aquatic environment might be responsible for creating free divalent ions hazardous elements that are routinely absorbed by fish gills and shows significant signs of aquatic pollution in respect of these heavy metals [4]. Certain inorganic fertilizers are commonly used in water culture and causes significant disturbance in natural environment of water dweller organisms. Most of agricultural water wastes that containing pesticides and fertilizers combines with industrial runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions in many water resources where fishes are cultivated. Moreover these heavy metals can be absorbed through their food chain that ultimately disturbs their normal physiological state [5]. Once accumulates in any medium in even trace levels may not be decomposed by passing hundreds of years and may shows critical effects to members of certain biota. Although the abundance of Fe, Zn and Cu in trace levels may not harm the organisms due to their biochemical role in the life processes of all aquatic plants and animals and considered to be essential metals in the aquatic and terrestrial environment in trace amounts [6].

Materials and Methods

Study Area

At North-East of Sindh Province, the study area (District Kashmore) is located which is lying at the border Punjab and Balouchistan Provinces. Water supply of Guddu barrage is the main source of irrigation of the District Kashmore. For Kashmore District fish is one of the popular products.

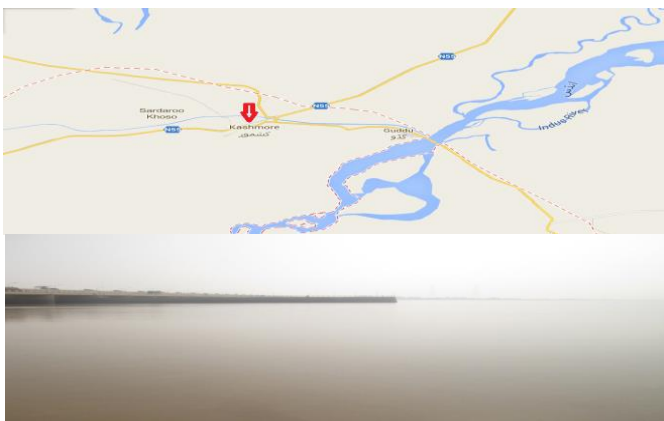


Fig 1: Map of the study area and photograph of Indus River.

Samples Description

Commonly available two kinds of fishes *Cirrhinus mrigala* and *Labeo rohita* matching with the standard size were collected from Indus River at Guddu barrage with help of professional fishermen available in the study area. The samples were ice cooled and stored in the laboratory at a temperature of -20°C until dissection and further analysis. The measurement of fish weight and length was carried out to the nearest grams and millimeters respectively. The collected samples were subjected to various tissues (liver, gills and liver) dissection followed by digestion prior to conduct elemental analysis [7-9].

Dried digestion method

In this method, 8g of dissected samples were taken from the biological specimen of both fishes. The samples were grinded by using grinder machine after proper drying in the oven followed by further drying in desiccators using active desiccant such as alumina. The biological specimens were further dried in desiccators using alumina as desiccant. The concentrated HNO_3 , conc. HClO_4 and H_2O_2 were treated with the dried samples in the ratio of 6:1:3 respectively. The colour of the samples was improved by heating at 130°C for one hour then filtered, cooled, diluted up to the mark with de-ionized distilled water and kept in the volumetric flasks for analysis of metals in gills and muscle tissue only. However, for the analysis of elements in liver tissues the samples were more digested to 25 ml so as to detect the lowest level of metals. The blank reagents were digested using same procedure as noted above. Same acid matrix was used for preparation of standard solutions [10-14].

Homogeneous Method of Digestion

In this method, dissected taken from the biological specimen of both fishes were homogenized and kept in a digestion tube of 300ml. The tri acids (hydrogen peroxide, Conc. Nitric Acid and Conc. HCl) used for digestion of samples were in a proportion of 3:2:1 as reported by Daziel and Baker, (1983). The colour of the samples was improved by heating at 130°C for one hour then filtered, cooled, diluted up to the mark with de-ionized distilled water and kept in the volumetric flasks for analysis of metals various tissues. The blank reagents were digested using same procedure as noted above [15-16].

Metals Analysis

The samples were analyzed for Elemental concentrations essential elements Na, K, Ca and Mn in the gills, liver and muscle tissues of each fish sample using Atomic Absorption Spectrophotometer (AA 100-Analyst, Perkin Elmer, USA) with air-acetylene flame mode. The samples were analyzed in triplicate and results were reported as microgram per gram ($\mu\text{g}\cdot\text{g}^{-1}$) units. Merck stock solutions were used to prepare standard solutions for each element. In patron of samples, the blank reagents were run in the instrument [17-20].

Results and Discussion

The results were expressed as mean of triplicate values. The whole data was analyzed statistically. The concentrations in various tissues of the fish species have been reported in Table-1 and Table-2 respectively.

Rohu (*Labeo rohita*)

The maximum concentration of sodium was noted in gills of Rohu fish sample FRG-1 with $6.37\mu\text{g}\cdot\text{g}^{-1}$ while minimum was found in FRG-4 with $0.75\mu\text{g}/\text{g}$ as mentioned Table 1. The highest level contents were noted in sample FRL-3 with $11.7\mu\text{g}\cdot\text{g}^{-1}$ while the lowest in FRL-1 with $0.97\mu\text{g}\cdot\text{g}^{-1}$ while in muscle of same fish species the maximum level of sodium found in sample FRM-3 with $6.64\mu\text{g}\cdot\text{g}^{-1}$ and minimum in sample FRM-2 with $0.30\mu\text{g}\cdot\text{g}^{-1}$. It has been observed that concentration of sodium was found high in samples followed by dried digestion method as compared to homogenous method in Gills, Liver and muscle Liver of Rohu fish.

The maximum amount Potassium was found in sample FRG-4 with $13.3\mu\text{g}\cdot\text{g}^{-1}$, while minimum concentration was observed in sample FRG-4 with $2.8\mu\text{g}\cdot\text{g}^{-1}$ as mentioned in Table 1. The highest amount was found in sample FRL-5 with $10.5\mu\text{g}/\text{g}$

and lowest in sample FRL-4 with 1.0 $\mu\text{g/g}$. The maximum concentration was detected in sample FRM-3 with 13.7 $\mu\text{g/g}$ and minimum was seen in sample No.FRM-2 with 2.0 $\mu\text{g-g}^{-1}$. It has been observed that concentration of Potassium was found high in samples treated by homogenous method as compared to dried method in Gills, liver and muscle samples of Rohu fish.

The highest level of Calcium was detected in sample FRG-3 with 49.5 $\mu\text{g-g}^{-1}$ and the lowest in sample FRG-1 with 0.52 $\mu\text{g-g}^{-1}$ as mentioned in Table 1. The maximum amount was observed in sample FRL-1 with 15.9 $\mu\text{g-g}^{-1}$ and the minimum was found in sample FRL-2 with 2.82 $\mu\text{g-g}^{-1}$. In case of muscle sample the highest level was noted in FRM-5 with 29.9 $\mu\text{g-g}^{-1}$ and the lowest was found in sample FRM-1 with 01.13 $\mu\text{g-g}^{-1}$. The amount of calcium was observed maximum in gills, liver and muscle of Rohu fish in samples treated by the homogenous method as compared to dried method.

The maximum amount of magnesium was found in sample FRG-4 with 17.5 $\mu\text{g-g}^{-1}$, while minimum in sample FRG-3 with 10.7 $\mu\text{g-g}^{-1}$ as mentioned in Table 1. While highest amount of magnesium was found in liver sample FRL-5 with 8.59 $\mu\text{g-g}^{-1}$ and lowest in sample FRL-4 with 3.67 $\mu\text{g-g}^{-1}$. The maximum concentration of magnesium was detected in muscle sample FRM-5 with 35.2 $\mu\text{g-g}^{-1}$ while minimum concentration was found in sample FRM-5 with 33.5 $\mu\text{g-g}^{-1}$. The amount of magnesium was detected high in samples of dried as compared to homogeneous samples in liver of Rohu.

Morakhi (*Cirrhinus mrigala*)

In gills of Morakhi fish the highest level of sodium was noted in sample FMG-5 with 6.16 $\mu\text{g-g}^{-1}$ while the lowest level was found in FMG-1 with the 1.60 $\mu\text{g-g}^{-1}$ as mentioned in Table 2. The highest concentration was found in the sample FML-5 with 8.48 $\mu\text{g-g}^{-1}$ and the lowest in sample FML-1 with 1.11 $\mu\text{g-g}^{-1}$. The maximum concentration was observed in the sample FMM-3 with 10.39 $\mu\text{g/g}$ while minimum in the sample FMM-1 with 1.60 $\mu\text{g/g}$. It is also observed that level of sodium was found maximum in gills, liver and muscle samples treated by dried method as compared to homogenous method in Morakhi fish.

The highest concentration of Potassium was found in sample FMG-3 with 3.62 $\mu\text{g-g}^{-1}$ while the lowest in sample FMG-3 with 0.30 $\mu\text{g-g}^{-1}$. The maximum level of potassium was observed in sample FML-3 with 5.7 $\mu\text{g/g}$ and minimum in sample FML-1 with 0.37 $\mu\text{g-g}^{-1}$. While highest level was noted in sample FMM-5 with 13.0 $\mu\text{g-g}^{-1}$ and lowest in sample FMM-2 with 2.5 $\mu\text{g-g}^{-1}$. It has been noted that concentration of Potassium was seen high in gills, liver and muscle samples treated by homogenous method as compared to dried method in Morakhi fish.

The highest concentration of calcium was observed in sample FMGH-3 with (22.7 $\mu\text{g-g}^{-1}$) while lowest in sample FMGD-3 with (1.65 $\mu\text{g-g}^{-1}$) as mentioned in Table 2. The highest amount was noted in sample FML-5 with (22.7 $\mu\text{g-g}^{-1}$) and lowest level was found in FML-1 with (1.15 $\mu\text{g-g}^{-1}$). In case of Muscle the maximum concentration was found in sample FMM-5 with (14.8 $\mu\text{g-g}^{-1}$) while lowest in sample FMM-3

with (6.15 $\mu\text{g-g}^{-1}$). It has been noted that concentration of Calcium was found high in Gills, liver and muscle of samples treated by homogenous method as compared to dried samples in Morakhi fish.

The highest concentration of magnesium was observed in sample FMG-4 with (17.3 $\mu\text{g-g}^{-1}$) while lowest in sample FMG-2 with (0.30 $\mu\text{g-g}^{-1}$) as mentioned in Table 2. The highest amount was noted in sample FML-3 with (8.62 $\mu\text{g-g}^{-1}$) and lowest level was found in FML-4 with (2.0 $\mu\text{g-g}^{-1}$). In case of Muscle the maximum concentration was found in sample FMM-5 with (37.3 $\mu\text{g-g}^{-1}$) while lowest in sample FMM-2 with (25.2 $\mu\text{g-g}^{-1}$). It has been noted that concentration of Magnesium was found high in Gills, liver and muscle of samples treated by dried method as compared to homogenous samples in Morakhi fish.

Average, minimum and maximum ranges of analytical data of biological specimens of Morakhi fish n=300 and Rohu fish n=300 are given in Table-3. It has been revealed from data that concentration of sodium, potassium, calcium and magnesium were found in range from (0.3-2.88, 8.40-13.7, 8.04-49.5 and 03.5-33.6 $\mu\text{g-g}^{-1}$) respectively in specimen of Rohu fish by Homogenous method. While by dried method the concentration were observed in ranges (1.62-11.7, 1.0-12, 0.52-9.85 and 5.3-35.4 $\mu\text{g-g}^{-1}$) for sodium, potassium, calcium and magnesium respectively.

However in case of *Cirrhinus mrigala* fish the concentration of sodium, potassium, calcium and magnesium were in range from (1.1-3.7, 0.3-13.0, 8.1-22.6 and 0.31-33.4 $\mu\text{g-g}^{-1}$) respectively for samples treated by homogenous method. While for dried method the levels were noted in range from (1.04-10.4, 0.3-35.7, 1.15-10 and 3.1-37.5 $\mu\text{g-g}^{-1}$) for sodium, potassium, calcium and magnesium respectively. The concentrations of sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were far below the recommended limit by WHO, US EPA and FAO.

The comparative studies of Level of sodium among Rohu and Morakhi fish specimen was evaluated as mentioned in Figure-2. It indicted trend of concentration of sodium in liver > muscle > gills of Rohu fish while in Morakhi fish samples muscle > liver > gills respectively.

The comparative studies of level of Potassium among Rohu and Morakhi fish specimen was evaluated as mentioned in Figure-3. It indicted trend of concentration of Potassium in muscle > gills > liver of Rohu fish while in Morakhi fish samples the level trend was seen muscle > liver > gills respectively. The comparative studies of Level of Calcium among Rohu and Morakhi fish specimen was evaluated as mentioned in Figure-4. It shows the trend of concentration of calcium gills > muscle > liver of Rohu fish while in Morakhi fish samples the concentration trend was seen liver > gills > muscle respectively.

The comparative studies of Magnesium level in Rohu and Morakhi fish specimen were exploited as given in Figure-5. It shows the trend of concentration of magnesium muscle > gills > liver of Rohu fish while in Morakhi fish samples the concentration trend was seen muscle > gills > liver respectively.

Table 1: Minimum, maximum and mean concentrations of metals in Rohu (*Labeo rohita*) fish samples by using homogeneous and dried methods of digestion

		Na $\mu\text{g}\cdot\text{g}^{-1}$						K $\mu\text{g}\cdot\text{g}^{-1}$					
		Homo digestion method n=10			Dried digestion method n=10			Homo digestion method n=10			Dried digestion method n=10		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Gills	FRG-1	1.55	1.59	1.57	6.35	6.39	6.37	8.40	8.45	8.43	11.3	11.9	11.6
	FRG-2	2.23	2.27	2.25	5.71	5.75	5.73	8.50	8.55	8.53	7.2	07.7	7.41
	FRG-3	1.55	1.59	1.57	6.14	6.17	6.15	8.61	8.65	8.63	4.4	04.9	4.67
	FRG-4	0.75	0.79	0.77	6.12	6.16	6.14	13.1	13.6	13.3	2.4	02.9	2.66
	FRG-5	0.94	0.99	0.962	4.62	4.66	4.64	12.4	12.8	12.6	2.5	2.91	02.7
Liver	FRL-1	0.95	0.99	0.97	8.22	8.26	8.24	8.70	8.75	8.73	8.4	8.82	8.58
	FRL-2	1.51	1.56	1.54	6.95	6.99	6.97	9.81	9.84	9.82	2.5	02.9	2.68
	FRL-3	2.21	2.26	2.23	11.7	11.76	11.7	8.42	8.45	8.43	3.1	3.6	3.37
	FRL-4	2.84	2.88	2.86	6.23	6.26	6.24	9.11	9.15	9.13	1.0	01.4	1.2
	FRL-5	02.0	2.05	2.03	9.35	9.39	9.37	10.5	10.9	10.7	3.2	03.5	3.35
Muscle	FRM-1	01.0	1.05	1.03	05.3	5.35	5.33	12.2	12.6	12.4	4.0	4.41	4.21
	FRM-2	0.30	0.35	0.33	1.62	1.66	1.64	9.42	9.9	9.65	2.0	02.4	2.22
	FRM-3	0.74	0.78	0.76	6.63	6.66	6.65	13.3	13.7	13.5	3.1	3.43	3.23
	FRM-4	1.13	1.17	1.15	6.13	6.17	6.15	8.42	8.9	8.63	3.2	03.6	3.38
	FRM-5	2.15	02.2	2.17	1.65	1.69	1.67	10.1	10.5	10.3	4.0	4.42	04.2

Table 2: Minimum, maximum and mean concentrations of metals in Rohu (*Labeo rohita*) fish samples using homogeneous and dried methods of digestion.

		Ca $\mu\text{g}\cdot\text{g}^{-1}$						Mg $\mu\text{g}\cdot\text{g}^{-1}$					
		Homo digestion method n=10			Dried digestion method n=10			Homo digestion method n=10			Dried digestion method n=10		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Gills	FRG-1	37.7	37.8	37.8	0.52	0.52	0.52	11.1	11.5	11.3	13.3	13.6	13.4
	FRG-2	37.7	37.7	37.8	1.15	1.15	1.15	14.1	14.5	14.3	16.2	16.6	16.4
	FRG-3	49.4	49.5	49.5	1.21	1.22	1.22	10.6	10.9	10.7	11.6	11.9	11.8
	FRG-4	10.0	10.04	10.0	1.85	1.85	1.85	15.2	15.6	15.4	17.3	17.6	17.5
	FRG-5	42.2	42.2	42.2	3.85	3.85	3.85	13.4	14.6	13.7	15.4	15.7	15.5
Liver	FRL-1	15.8	15.9	15.8	0.70	0.74	0.72	06.0	06.4	6.20	08.2	08.5	8.33
	FRL-2	14.8	14.9	14.8	2.82	2.85	2.83	5.31	5.71	5.51	7.21	07.5	7.35
	FRL-3	8.04	8.08	8.06	5.65	5.68	5.67	4.32	04.7	04.5	6.42	6.81	6.57
	FRL-4	8.05	8.08	8.06	5.65	5.68	5.66	03.5	03.9	3.67	05.3	05.7	5.5
	FRL-5	8.84	8.87	8.85	9.82	9.85	9.84	6.51	6.81	6.63	8.41	8.81	8.59
Muscle	FRM-1	14.5	14.5	14.6	1.13	1.16	1.15	25.2	25.6	25.4	27.5	27.9	27.7
	FRM-2	14.5	14.6	14.6	1.41	1.45	1.43	26.4	26.8	26.6	28.4	28.8	28.6
	FRM-3	8.56	8.59	8.57	1.43	1.46	1.45	29.0	29.4	29.2	32.0	32.4	32.2
	FRM-4	22.2	22.2	22.2	8.32	8.35	8.34	31.5	31.9	31.7	33.4	33.8	33.6
	FRM-5	29.9	29.9	29.9	7.57	7.68	7.65	33.3	33.6	33.5	35.0	35.4	35.2

Table 3: Minimum, maximum and mean concentrations of metals in Morakhi (*Cirrhinus mrigala*) fish samples using homogeneous and dried methods of digestion

		Na $\mu\text{g}\cdot\text{g}^{-1}$						K $\mu\text{g}\cdot\text{g}^{-1}$					
		Homo digestion method n=10			Dried digestion method n=10			Homo digestion method n=10			Dried digestion method n=10		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Gills	FMG-1	1.59	1.66	1.63	5.52	5.57	5.545	2.40	2.90	2.65	0.91	0.95	0.93
	FMG-2	2.18	2.25	2.22	4.84	4.89	4.866	2.20	2.61	2.46	2.91	2.95	2.93
	FMG-3	1.92	1.96	1.94	1.04	1.08	1.063	03.2	3.62	3.40	0.30	0.35	0.33
	FMG-4	1.61	1.66	1.64	4.35	4.39	4.37	02.0	2.50	2.25	0.95	0.99	0.97
	FMG-5	2.01	2.05	2.03	6.11	6.16	6.135	01.9	1.95	1.92	0.31	0.36	0.33
Liver	FML-1	1.11	01.6	1.35	5.11	5.15	5.129	0.30	0.35	0.33	0.32	0.37	0.35
	FML-2	1.50	01.9	1.69	6.85	6.89	6.868	3.81	3.85	3.83	1.34	1.39	1.36
	FML-3	2.40	2.81	2.62	6.34	6.39	6.369	5.72	5.75	5.73	0.94	0.99	0.96
	FML-4	3.22	3.62	3.4	6.94	6.98	6.959	3.53	3.55	3.53	2.72	2.69	2.67
	FML-5	3.21	03.7	3.45	8.44	8.48	8.464	03.0	03.5	3.25	2.64	2.69	2.67
Muscle	FMM-1	1.57	1.62	1.59	4.44	4.48	4.46	8.80	8.85	8.83	3.55	3.61	3.50
	FMM-2	1.75	1.79	1.77	3.81	3.85	3.831	9.65	9.69	9.67	2.32	2.82	2.55
	FMM-3	2.01	2.05	2.03	10.35	10.39	10.37	11.7	11.7	11.7	11.3	11.7	11.5
	FMM-4	02.3	2.35	2.33	7.95	7.99	7.968	12.4	12.4	12.4	35.4	35.7	35.6
	FMM-5	1.71	1.75	1.73	2.84	2.97	2.879	13.0	13.0	13.0	4.11	4.50	4.30

Table 4: Minimum, maximum and mean concentrations of metals in Morakhi (*Cirrhinius mrigala*) fish samples using homogeneous and dried methods of digestion

		Ca $\mu\text{g}\cdot\text{g}^{-1}$						Mg $\mu\text{g}\cdot\text{g}^{-1}$					
		Homo digestion method n=10			Dried digestion method n=10			Homo digestion method n=10			Dried digestion method n=10		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Gills	FMG-1	22.4	22.5	22.4	2.75	2.79	2.77	12.0	12.4	12.2	14.0	14.4	14.2
	FMG-2	20.3	20.3	20.3	2.12	2.16	2.14	0.30	10.4	9.21	11.5	11.9	11.7
	FMG-3	22.7	22.7	22.6	1.65	1.69	1.67	11.2	11.5	11.34	14.2	14.5	14.4
	FMG-4	11.9	11.9	11.9	3.10	3.15	3.12	15.0	15.0	15.02	17.1	17.5	17.3
	FMG-5	18.5	18.5	18.5	1.72	1.76	1.74	13.1	13.4	13.27	15.2	15.5	15.3
Liver	FML-1	13.0	13.0	13.0	1.15	1.19	1.17	07.0	7.42	7.18	7.30	07.7	7.50
	FML-2	15.8	15.8	15.9	3.13	3.17	3.16	05.0	5.43	5.19	06.3	6.71	6.51
	FML-3	15.8	15.8	15.9	4.63	4.67	4.65	08.0	8.45	8.21	08.2	8.62	8.42
	FML-4	16.4	16.5	16.4	5.83	5.87	5.85	2.01	2.40	02.2	3.14	3.50	3.34
	FML-5	22.6	22.7	22.7	4.13	4.18	4.16	5.02	5.43	5.22	5.22	5.61	5.45
Muscle	FMM-1	8.10	8.14	8.12	8.64	8.68	8.66	32.0	32.4	32.2	36.0	36.4	36.2
	FMM-2	8.61	8.64	8.62	6.64	6.69	6.66	25.0	25.4	25.2	27.0	27.5	27.3
	FMM-3	8.62	8.64	8.62	6.15	6.19	6.17	27.0	27.4	27.2	31.0	31.4	31.2
	FMM-4	14.4	14.8	14.6	10.0	10.0	10.0	28.0	28.4	28.2	32.0	32.4	32.2
	FMM-5	14.4	14.8	14.6	08.5	8.56	8.53	33.0	33.4	33.2	37.0	37.5	37.3

Table 5: Average ranges of obtained results of the biological specimen of both the fish samples

	Recommended values WHO (2004)	Rohu (<i>Labeo rohita</i>) n=300*						Morakhi (<i>Cirrhinius mrigala</i>) n=300 ^a					
		Homo n=150*			Dried n=150*			Homo n=150*			Dried n=150*		
		Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.	Min.	Max.	Ave.
Na	12-1500mg/per day	0.3	2.88	1.48	1.62	11.7	6.20	1.10	03.7	02.1	1.04	10.4	5.69
K	3000-4500mg/per day	8.40	13.7	10.2	01.0	11.9	4.36	0.30	13.0	5.66	0.30	35.7	4.73
Ca	800-1300mg/per day	8.04	49.5	21.5	0.52	9.85	3.55	08.1	22.6	15.6	1.15	10.0	4.66
Mg	400mg/per day	03.5	33.6	16.0	5.30	35.4	17.8	0.31	33.4	15.7	3.11	37.5	17.8

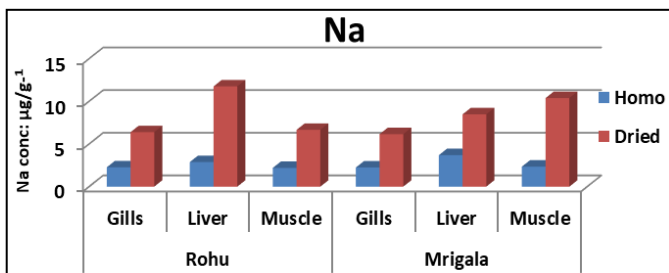


Fig 2: Average sodium concentrations in various organs of Rohu and Mrigala fish samples

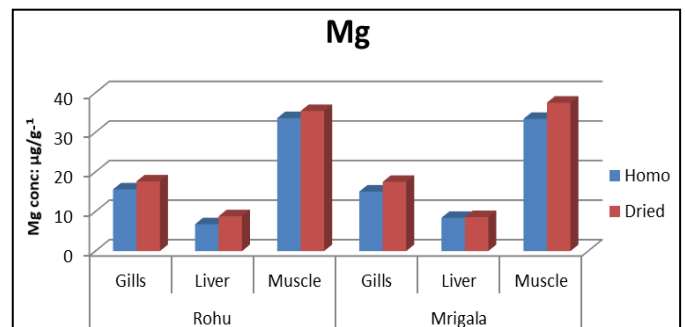


Fig 5: Average magnesium concentration in various organs of Rohu and Mrigala fish samples.

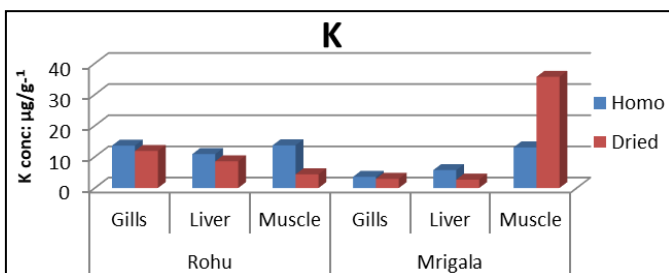


Fig 3: Average potassium concentrations in various organs of Rohu and Mrigala fish samples.

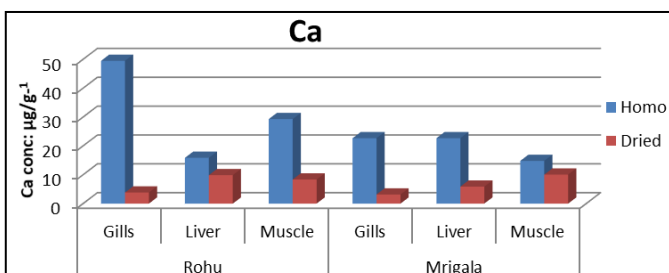


Fig 4: Average calcium concentrations in various organs of Rohu and Mrigala fish samples.

Conclusion

In present study level of essential elements such as sodium, potassium, calcium and magnesium in gills, liver and muscle tissues of commonly consumable fishes (Rohu and Mrigala) of the study area were investigated. The concentration order of essential metals was noted in homogeneous samples $\text{Mg} > \text{Ca} > \text{K} > \text{Na}$ and in dried samples $\text{Mg} > \text{Na} > \text{K} > \text{Ca}$ in *Labeo rohita* fish samples. While order of concentration was found in homogeneous samples $\text{Mg} > \text{Ca} > \text{K} > \text{Na}$ and in dried samples $\text{Mg} > \text{K} > \text{Ca} > \text{Na}$ accordingly (*Cirrhinius mrigala*) fish samples. It was concluded that the concentration of essential elements found were far below the recommended limit by WHO, US EPA and FAO possibly due to enough quantity of freshwater in Indus River. So it is not a good indication for consumers of above noted fish species as the required essential nutrients cannot be completed only eating these fishes. For essential elements, only relying on these fishes, the residents of the study area and the adjoining areas might face essential elements deficiencies in the body.

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Conflicts

The authors have not declared any conflicts of interest.

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